Dissertation on

"A STUDY OF CHROMOSOMAL ABNORMALITIES IN LEUKEMIC PATIENTS IN A TERTIARY CARE HOSPITAL"

Submitted in partial fulfillment for the Degree of

M.D PATHOLOGY BRANCH – III

THE TAMIL NADU DR.M.G.R MEDICAL UNIVERSITY CHENNAI

INSTITUTE OF PATHOLOGY MADRAS MEDICAL COLLEGE

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CERTIFICATE

This is to certify that this dissertation entitled "**A STUDY OF CHROMOSOMAL ABNORMALITIES IN LEUKEMIC PATIENTS IN A TERTIARY CARE HOSPITAL**" is the original work of **Dr.RAMESH.B.** in partial fulfilment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R. Medical University to be held in May 2019.

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DECLARATION

I, **Dr.RAMESH. B,** solemnly declare that the dissertation titled " **A STUDY OF CHROMOSOMAL ABNORMALITIES IN LEUKEMIC PATIENTS IN A TERTIARY CARE HOSPITAL**" is the bonafide work done by me at the Institute of pathology, Madras Medical College under the expert guidance and supervision of **Prof.Dr.SELVAMBIGAI M.D.Dch,** Professor of Pathology, Institute of pathology, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University towards partial fulfilment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

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CERTIFICATE OF APPROVAL

To
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Dear Dr.Ramesh.B.,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY OF CHROMOSOMAL ABNORMALITIES IN LEUKEMIC PATIENTS IN A TERTIARY CARE HOSPITAL" - NO.22122017**

The following members of Ethics Committee were present in the meeting hold on **05.12.2017** conducted at Madras Medical College, Chennai 3

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We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

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ABBREVIATION

AML	: Acute myeloid leukemia
ALL	: Acute lymphoid leukemia
CML	: Chronic myeloid leukemia
CLL	: Chronic lymphoid leukemia
FISH	: Flourescent in situ hybridisation
GM-CSF	: Granulocyte monocyte colony stimulating factor
CML-BP	: Chronic myeloid leukemia- blast phase
MPO	: Myeloperoxidase
MDS	: Myelodysplastic syndrome
PBS	: Phosphate buffer saline
CGH	: Comparative genomic hybridization
SKY	: Spectral karyotyping
PHA	: Phytohemagglutinin
WBC	: White blood corpuscules

TABLE OF CONTENTS

INTRODUCTION

Leukemias are hematopoietic malignant neoplasms arising by the clonal expansion of the hematopoietic stem cell and can be divided into acute and chronic leukemias [1]. They can be broadly divided into lymphoid and myeloid, on the basis of the cell lineage from which they arise and give rise to. They can additionally be subdivided into four major types, acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL) [2]. Acute leukemia shows a preponderance of immature blast cells, while chronic leukemia, either myeloid or lymphoid is characterized by a preponderance of mature cells (most often) leading to the accumulation of abnormal cells in the bone marrow and the peripheral blood [3]. The myeloid lineage includes the population of granulocytes, monocytes, thrombocytes and erythrocytes whereas the lymphoid lineage differentiates into B- and T- lymphocytes [4,5]. The prototype of chromosomal aberrations was first elucidated in CML, where a translocation between chromosomes 9 and 22, the Philadelphia chromosome is identified and the fusion protein thus formed (BCR/ABL) shows constitutive tyrosine kinase activity [6]. ALL is characterized by clonal excess of lymphoblasts in the bone marrow and mainly affects children, likewise AML is characterized by clonal excess of myeloblasts,

affecting mainly the adults [7]. The origin or the development of Leukemia is a complex multistep development involving numerous chromosomal aberrations like mutations, insertions, deletions, translocations, amplifications or epigenetic changes [8]. These aberrations occur in either the oncogenes, the function of which is controlling the cell proliferation or in the tumor suppressor genes, which has a pivotal role in preventing the excess cell proliferation and differentiation or in the genes, which control the DNA repair and apoptosis. A single chromosomal aberration may be an initiating event in the leukemogenesis, which further promotes the acquisition of numerous other mutations to accumulate, thus leading to the progression of the disease. Complex genetic aberration has an implication in the prognosis and treatment. Arriving at the diagnosis of acute myeloid or lymphoid leukemia, based on only the morphology of the immature cells may be misleading, as some lymphoblast can have nucleoli and indented nucleus, whereas some myeloblasts can have an indented nuclei and scant cytoplasm. The detection of the chromosomal aberrations in such cases may be of extreme help in establishing the exact diagnosis and classifying the patients based on the karyotype into risk groups, thus providing the vital information regarding the prognosis of the patient. Cryptic aberrations may not be identified by conventional karyotyping technique. In that case, FISH, spectral karyotyping can be resorted.

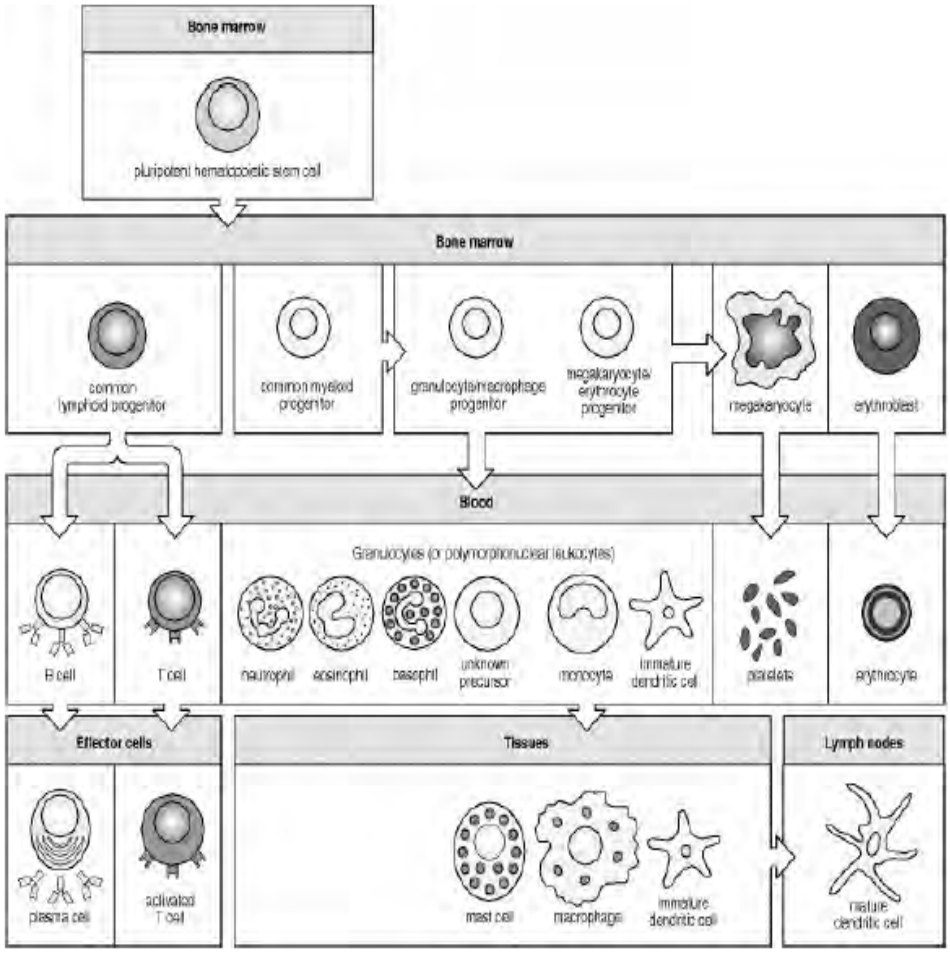
AIMS AND OBJECTIVES

- To classify acute and chronic leukemia based on morphology in peripheral smears stained by leishman stain.
- To determine the chromosomal abnormality associated with each type of leukemia
- To study the distribution of karyotype, in relation to the hematological parameters like total leucocyte count, platelet count and hemoglobin levels.

REVIEW OF LITERATURE

Blood cell formation:

Hematopoiesis or blood cell formation takes place primarily in the bone marrow. All the elements of blood and lymph are derived from pleuripotent hematopoietic stem cell. Pleuripotent stem cells are present in low numbers in the bone marrow. Each of them have the potential to proliferate and differentiate, giving rise to all myeloid blood and and white blood cells, as well as to erythrocytes and platelets. However, under normal circumstances, the vast majority of the pleuripotent stem cells are in a quiescent state, and only a few are active in the process of blood cell formation. The processes of self replication and rejuvanation, differentiation to a more restricted phenotype and cell death are regulated very strictly by molecular mechanisms and regulatory cytokines. These regulation processes concerns also about the more mature offspring of the stem cell e.g. the committed progenitor cell, whose developmental lineage already shows restriction to one lineage, but is still capable of self renewal. The regulatory mechanisms are not yet fully known, but are thought to be mandatory for understanding normal hematopoiesis as well as the etiology of leukemia (Sawyers et al 1991).



In the last few years, several soluble factors have been identified, which are involved in the regulation process e.g. growth factors, and small peptides that are produced by blood cells or by bone marrow stromal cells. GM-, G-, M-CSF and several other interleukins such as ll-1, 3 and 6 have been identified as stimulators of bone marrow stem cells and committed precursor cells, thus acting as positive regulator. Examples of negative regulators (inhibitors) of these cells include transforming growth factor Jl (TGF Jl), which is produced by the marrow stromal cells and small peptides like stem cell inhibitor (SCI), which is

produced by macrophages (Graham et a!, 1990, Zebo et al,1990, Williams et al, 1990, Huang et al !990, Dexter et al, 1977, Clark et al, 1987).

In addition to these factors , cell to cell contact and specific effects of the extracellular matrix play a role in regulation of stem cell function, the latter possibly by interaction with other growth factors (Roberts eta!, 1988). Any disturbances in these regulatory mechanisms might result in uncontrolled cell proliferation and or failure of the progenitor cells to differentiate into mature cells.

Blood cancer:

Blood cancer or leukemia comprises a heterogeneous group of diseases resulting from neoplastic transformation of hematopoietic cells. The main characteristics are uncontrolled proliferation of hematopoietic cells, that in most cases, do not retain their capacity to differentiate normally into mature blood cells. This differentiation arrest can occur in every stage of maturation and in every cell lineage of blood cell differentiation, resulting in distinct forms of leukemia. Several attempts have been made to formulate a classification system for the different types of leukemia, in the past years. Based on the degree of maturation of the cells, the leukemias are classified as acute or chronic. A further subclassification is made according to the predominant cell lineage

affected, e.g. lymphoid, myelogenous, or monocytic and to morphological and immunological characteristics of the leukemic cells.

ACUTE MYELOID LEUKEMIA

EPIDEMIOLOGY:

Acute myeloid leukemia is the most common leukemia in adults, ranging for approximately 80% [9].Within the United States, AML occurs in three to five per 100 000 population [10]. The incidence of AML is ~ 1.3 per 100 000 population in patients less than 65 years old, whereas it is 12.2 cases per 100 000 population in those over 65 years, thus implying that the incidence of AML increases with age[11]. In India, of all the acute and chronic leukemias, the incidence of AML ranges between 20 - 25%. The AML occurs at a median age of 40 years in the developing countries population [12]. AML constitutes only 15% to 20% of cases in patients of approximately 15 years of age [13].

ETIOLOGY:

There are numerous environmental and genetic risk factors contributing to the development of AML. Some of the environmental factors include occupational exposure to organic solvents such as benzene and cigarette smoking (particularly FAB-M2) [14,15]. Therapy related AML, a category of AML occurs secondary to chemotherapy for

other types of tumors, including the solid tumors (t-AML) [16]. Secondary AML is another category of AML which may arise from myelodysplastic syndrome (MDS) or myeloproliferative disorders. Genetic disorders include Down syndrome or genomic instability syndromes like Fanconi anemia, or Bloom syndrome predisposes to AML [17, 18, 19, 20]. Another important independent prognostic factor is the age of the patient, with the older patients having an adverse prognosis, since the high-risk cytogenetics are frequently observed in them [21, 22]. 50-80% of the population exhibit acquired clonal somatic mutations [23, 24]. Regardless of its etiology, the pathogenesis of AML involves the abnormal proliferation and differentiation of a clonal population of myeloid stem cells.

CLINICAL FEATURES:

The accumulation of malignant myeloid cells within the bone marrow and infrequently in other organs including soft tissues, contributes for most of the clinical manifestations of AML. The majority of patients present with a leukocytosis, anemia and thrombocytopenia due to the suppression of other lineages by the proliferating blasts. The common complaints include fatigue, anorexia and weight loss [25]; lymphadenopathy and splenomegaly may be present. Some patients can present with a subcutaneous swelling (chloroma) in the very early stage of the disease even before the blasts are evident in the

peripheral blood, whereas in the late stages, they develop bone marrow failure symptoms of anemia and thrombocytopenia[26]. Gum bleeding and purpuric hemorrhages may also be the presentation in some patients due to infiltration of leukemic cells in gum and skin (leukemia cutis).

CLASSIFICATION:

In 1976, the French–American–British classification system was first established to distinguish between different types of AML. Based on the morphological and cyto-chemical characteristics of the leukemic cells, it describes AML as eight distinct subtypes (M0 through M7) [26, 27]. In 2001, the World Health Organization (WHO) introduced a new classification system for AML by incorporating the molecular testing for detecting some mutations, thus for moving towards an improvised diagnosis and better management of the patients, which was later revised and a new version was released in 2008. Later in 2016, a new revised version was released by the WHO by incorporating additional genetic mutations observed in AML which may have a significant influence and impact on the patient management, in the future, includes six major disease entities:

- AML with recurrent genetic abnormalities
- AML with myelodysplasia-related features

- Therapy related AML
- AML not otherwise specified
- Myeloid sarcoma
- Myeloid proliferation related to Down syndrome

The category of AML with recurrent genetic abnormalities has 11 subtypes based on distinct chromosomal translocations, they exhibit. The diagnosis of AML can be established even in the absence of 20% blasts in peripheral smear/bone marrow if the t(8;21), inv(16) or t(15;17) are documented [28]. In addition, the provisional entities AML with mutated NPM1 and AML with mutated CEBPA which were introduced as a part of the 2008 revision were modified in 2016 classification by claasifying them as a separate entity of AML with mutated genes and the entities like AML with BCR-ABL1 and AML with mutated RUNX1 were also introduced as part of the 2016 revision [29]. The diagnosis of AML with myelodysplasia-related changes can be made if the following features are present. This includes:

- History of MDS
- Morphological evidence of dysplasia in two or more myeloid cell lineages
- The presence of myelodysplasia-related cytogenetic abnormalities such as monosomy 5 or 7, and deletion 5q or 7q [30].

CHROMOSOMAL ALTERATIONS:

More than 40 years ago, in a pioneering work of Janet Rowley and others, recurrent cytogenetic alterations, i.e. structural or numerical chromosomal abnormalities in AML were described. The first translocation to be described in human cancers was the discovery of recurring balanced translocations between chromosomes 8 and 21, termed t(8;21) (q22;q22) and this laid a platform for understanding of cancer genetics and it turned to be a milestone in genomics of malignancies [31]. There are numerous cytogenetic aberrations described in AML and there exist a great diversity in the cytogenetic aberrations among the patients of AML. Approximately 50-60% of AML patients have abnormal karyotypes, whereas other patients have normal karyotypes. Despite the complex nature of the cytogenetic abnormalities in AML patients, various studies were done to categorise the cytogenetic abnormalities based on prognostication. This lead to the classification 29 established by the European Leukemia Network (ELN) and Medical Research Council (MRC). This is as follows:

Favorable Risk
t(15;17)(q22;q21) inv(16)(p13.1q22); t(16;16)(p13.1;q22) t(8;21)(q22;q22)
Intermediate Risk
Normal karyotype Cytogenetic abnormalities not classified as favorable or adverse
Adverse Risk
abnormal(3q), excluding t(3;5)(q21~25;q31~35) inv(3)(q21q26.2); t(3;3)(q21;q26.2) add(5q), del(5q), -5 -7, add(7q)/del(7q) t(6;11)(q27;q23) t(10;11)(p11~13;q23) t(11q23), excluding t(9;11)(p21~22;q23) and t(11;19)(q23;p13) t(9;22)(q34;q11) -17/abnormal(17p) complex karyotype*

*Defined as >4 independent chromosomal aberrations

The largest group is the one with intermediate risk exhibiting cytogenetically normal karyotype (CN-AML), contributing upto 40-50% of patients with de novo AML [32] [33]. This group is however, is heterogeneous with some patients achieving complete remission and lead a longer life, while others present with an aggressive and even resistant disease with higher number of relapse rates.

Aneuploidy, i.e. gain or loss of entire chromosome, occurs due to a defective chromosome segregation during mitosis [34]. The chromosomal aberrations affect diverse gene loci in the chromosomes. So, it is a great

task to understand the direct effect of such aberrations on the leukemogenic process. However, the gene dosage plays a pivotal role in the leukemogenesis as described in a study of 80 patients with trisomy 8 (+8) as the sole aberration, 452 genes were significantly upregulated and 329 downregulated in +8 AML compared to cytogenetically normal AML [35]. Of the 452 upregulated genes, 189 (42%) were located on chromosome 8. The precise molecular mechanism which causes these chromosomal abnormalities still remains an enigma. Studies have shown that the homologous recombination, non-homologous end joining and chromosome fragile sites are the potential triggers for the formation of translocations [36]. Moreover, it was shown that during mitosis, chromosomal segregations may as well lead to the occurrence of translocations [37]. In general, translocations lead either to the formation of new fusion genes [38, 39] or causes the juxtaposition of regulatory elements from one translocation partner to the other, resulting in aberrant gene expression [40, 41]. The functional consequences of many chromosomal rearrangements have been subject to intensive studies. The recurrent translocation t(8;21)(q22;q22), for example, leads to the formation of chimeric *RUNX1/RUNX1T1* gene (also known as *AML1-ETO*) [42]. RUNX1 is an important transcription factor which plays an important role in the regulation of hematopoiesis [43, 44] and forms a part of the so-called core binding factor (CBF) complex. As a result of the

fusion with RUNX1T1, the normal function of RUNX1 in the CBF complex is altered, thus preventing the transcription of CBF target genes important for myeloid differentiation, and thereby leading to the disruption of normal hematopoiesis and inactivation of tumor suppressor genes [45,46]. Translocations, inversions and deletions are common in AML. Mutations in *NPM1*, *CEBPA* and *FLT3* genes have been introduced as a separate group as prognostic markers into the classification of risk groups [47].

LABORATORY FINDINGS:

The diagnosis of acute leukemia is established by the presence of 20% or more myeloblasts in the bone marrow or peripheral blood. In the presence of the t(8,21), inv(16) and t(15,17), the blast count less than 20% can be considered as diagnostic of AML[47]. AML can further be subdivided by demonstrating the myeloid origin of these cells through testing for myeloperoxidase activity, immunophenotyping or demonstrating the presence of Auer rods. The latter finding consists of azurophilic, often needle-shaped cytoplasmic inclusions of the contents of the primary granules that are commonly seen in APL, acute myelomonocytic leukemia and the majority of AML with t(8;21). The diagnosis of AML can also be established in the presence of an extramedullary tissue infiltrate.

ACUTE LYMPHOID LEUKEMIA

EPIDEMIOLOGY:

The worldwide annual incidence of adult acute lymphoblastic leukemia (ALL) is about one in 100,000 poulation [48]. In India, among the leukemic patients, the incidence of ALL is 15-25%. It is more common in children when compared to adults. The children respond well to chemotherapy and approximately 95% achieve complete remission with induction chemotherapy and 75-80% never relapse 85%. Although 85% of the adult patients perform well with chemotherapy, the relapse and recurrence rates are higher [49]. It is vital to distinguish AML from ALL, as the treatment protocol and strategies of management differs [50].

CLINICAL FEATURES:

Fever is the most common presenting complaint [51]. Other presentations include anemia, thrombocytopenia, infections as a result of the diminished production of normal WBCs. Mediastinal involvement may present as symptoms of dyspnea, SVC syndrome. CNS involvement and other organ involvement such as hepatomegaly, splenomegaly and lymphadenopathy are more common in ALL when compared to AML [51]. The clinical parameters like age, sex, WBC count, bulky extramedullary disease and CNS involvement are prognostically important [52]. The

distinction between B-ALL and T-ALL by immunophenotyping helps in predicting the prognosis, as the mediastinal involvement are more common with T-ALL [53] and this subcategorisation also has a role in the management of the patients.

CHROMOSOMAL ABNORMALITIES:

With the relavant clinical data and laboratory examination findings, Karyotyping yields an additional information of significant prognostic implication and provides a more personalized approach for treatment strategy. Most of the ALL cases are detected to have only a single chromosomal abnormality on the routine G-banding technique and karyotyping still remains the gold standard basic investigation in assessing the chromosomal abnormalities in these patients [54]. Chromosomal abnormalities are detected in 65-70% of ALL patients with conventional karyotyping [55]. These include numerous numerical and structural abnormalities. The numerical abnormalities include high hyperdiploidy, moderate hyperdiploidy and hypodiploidy. Out of the numerous structural aberrations found in ALL, most of them are translocations. Some of the most common translocations include t(9;22) (q34;q11), t(12;21) (p13;q22), t(1;19) (q23;p13), MLL rearrangements with t(4;11) (q21;q23) and t(11;19) (q23;p13) as the most common and intrachromosomal amplification of 21q [56]. There are numerous structural

aberrations detected in T-ALL, but the prognostic significance of many such aberrations have not been established [57, 58, 59]. The adults with ALL carry a worst prognosis than children because the aberrations associated with an unfavourable prognosis are common in adults when compared to children [60].

NUMERICAL ABERRATIONS:

High hyperdiploidy:

High hyperdiploidy in ALL is defined as 52-60 chromosomes and is characterized by gain of chromosomes as multiple non random trisomies and tetrasomies. It occurs in approximately 30% of B-precursor ALL in children and 2-11% of adult B-precursor ALL. This aberration is associated with low-risk features and is associated with a favourable prognosis [61]. The most common chromosomal gains are chromosomes X, 4, 6, 10, 14, 17, 18 and 21 [62, 63, 64]. The gain of chromosomes 4, 10, 6 and 17 is associated with a particularly favourable prognosis whereas a gain of chromosome 5 and i(17q) have been associated with a poor prognosis [65].

Hypodiploidy:

Hypodiploidy is characterized by less than 45 chromosomes by loss of chromosomes. In particular, near-haploidy (24-28 chromosomes) is associated with an extremely poor prognosis in both children and adults [66].

Moderate hyperdiploidy:

It has been observed in around 75% of the cases. This is associated with intermediate prognosis [67]. Most cases have been observed to have 47 chromosomes while few have 48 chromosomes. The most common chromosome gains are +21, +X, +8 and +10.

STRUCTURAL ABERRATIONS:

ETV6-RUNX1 rearrangement:

The t(12;21)(p13;q22) results in ETV6/RUNX1 rearrangement. The gene ETV6 encodes an ETS family transcription factor and fuses with RUNX1 which encodes a transcription factor with a DNA binding domain. The t(12;21) is the most frequent structural aberration in childhood ALL and it contributes to 25% of the childhood ALL cases [68], but it is only rarely found in adults (3-5%). Age between 2 and 5 years, and a low WBC count are the favourable prognostic factors associated

with this aberration. Few earlier studies have shown this aberration to be associated with a favourable prognosis [69, 70] although later studies reveal it to have no survival benefit [71, 72] and late relapses are quite frequent. In addition, 50-70% of the patients have been detected to have loss of 12p and gain of chromosome 21 as the most common, identified in almost 30% of the patients. As 12p13 deletions occur at an increased frequency, in about 50% of the patients with ETV6/RUNX1 translocation, it has been hypothesized to represent an important secondary event in the progression of leukemia [73]. RUNX1 is a common translocation partner in AML, as well, e.g. t(8;21), t(3;21) and rarely t(16;21) and t(19;21) 74, but the only translocation involving RUNX1 in ALL is t(12;21)(p13;q22).

BCR/ABL1 Rearrangement:

The t(9;22)(q34;q31) is found in 25% of the adults. It occurs at a lower frequency in children and is found in only 3% of the children. This translocation is almost always associated with a poor prognosis, irrespective of whether it occurs in children or adults [53]. The BCR/ABL fusion protein acts as a constitutively active oncoprotein. It acts by switching on several signaling pathways thereby contributing to the malignant transformation [75]. The size of the BCR fusion proteins varies as the splicing of the chromosome occur at different breakpoint regions

in the BCR locus. Three different protein products have been described: p190, p210 and p230. This contributes to the varied biological, clinical phenotypes and variable presentation associated with the BCR/ABL1 variants[76]. In children with ALL, P190 is the most frequent oncoprotein generated and it contributes about 80% to 90% of all Philadelphia positive ALL. In adults, the distribution of P190 and P210 are equal [77]. The difference in the BCR breakpoints also establishes the stemcell origin of some leukemias. The cases with a P210 BCR/ABL1 fusion originate in a CD34+ CD38- CD19- stemcell population corresponding to a lymphomyeloid hematopoietic stemcell. In contrast, ALL with a P190 BCR/ABL1 arises in a progenitor cell committed to a B cell lineage [78]. Nowadays, patients with both the breakpoints are regarded as a single entity and treated with the same protocols. However, a study indicated a better prognosis in P190 BCR/ABL1 ALL [79].

PBX1/TCF3 rearrangement:

t(1;19)(q23;p13) leads to the fusion of TCF3 (E2A) gene on chromosome 19p13 to the PBX1 gene on 1q23. This occurs in a balanced and unbalanced form, which arises from non-disjunction leading to loss of the chromosome 1 [80, 81]. This aberration is found in 5% of children and <5% of adults with ALL [82]. It is associated with a good prognosis in

children and the 5 year survival rate is 92% in children [83]. It is associated with a relatively poor prognosis in adults [84].

MLL (Mixed lineage leukemia gene rearrangements):

Rearrangements of the MLL gene at 11q23 are found in 75% of infants with ALL. t(4;11)(q21;q23) is the most common structural aberration found in ALL infants. They are also found in older children and adults, but at a lower frequency (2% and 7% respectively) [85]. The prognosis is poor in infants and adults but better in older children [86, 87]. It has been hypothesized that there is prenatal origin with this translocation as in most of the cases, the translocations are present from birth [88, 89]. MLL gene rearrangement as the name suggests are found both in ALL and AML and in a proportion of mixed lineage leukemia [90].

Other recurrent structural abnormalities:

Other recurrent structural abnormalities in BCP-ALL includes t(9;20)(p13;p11), t(9;12)(p13;q13), t(7;9)(p11;q11), t(12;17)(p13;q12) and t(8;14)(q11.2q32) [91,92]. These aberrations occur in 2% of the individuals with ALL and their significance has not been established.

T-ALL:

This subtype is not very common in children with ALL. Only approximately 12% of ALL in childhood has been estimated to be of T-cell lineage [93]. It is most common in boys when compared to girls and more common in older children and young adults. This disease is characterized by high WBC count, lymphadenopathy, splenomegaly, mediastinal mass and CNS involvement and it is aggressive and associated with a poor prognosis [94]. The cytogenetics and molecular genetics of T-ALL is poorly understood when compared to B-ALL. The genetic studies have lead to the identification of large number of translocations, deletions, amplifications and mutations. Activating mutations of NOTCH1 genes have been observed in 50% of the T-ALLs [94]. One common finding in T-ALL is deletion of 9p21.3. Cryptic deletions have been identified in 60% of children and adults with T-ALL.

Outcome	Karyotype	Immunophenotype
Favorable	Hyperdiploid: >50 chromosomes	CALLA (80%)
Intermediate	Normal	
Poor	Translocations	
	t(1;19)	Pre-B
	t(8;14) (q24;q11)	T-ALL
	t(11;14) (p13;q11)	T-ALL
	inv(14)	
Very poor	t(4;11)	0-ALL
	t(9;22)	CALLA/Pre-B
	t(8;14) (q24;q32)	B-ALL
	near haploid	
Unclear	6q−	No specific phenotype
	del/t 9p	
	del/t 12p	

CALLA, common acute lymphoblastic leukemia antigen; T-ALL, T-cell acute lymphoblastic leukemia; 0-ALL, Null-acute lymphoblastic leukemia; B-ALL, B-cell acute lymphoblastic leukemia

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Normal Karyotype in ALL:

The major problem with the peripheral blood karyotyping is the yield of metaphases, which are often of a poor quality and the preferential growth of non-malignant cells may lead to false negative normal karyotypes [95]. Minute translocations may also remain undetected because they are below the resolution of chromosome banding. Thus, the normal karyotype patients have numerous genetic changes and consequently, the prognostic or diagnostic significance of many of the chromosomal aberrations have not been reliably established, leading to under- or over estimation of the relapse risk [96, 97]. This implies the further genetic testing by FISH and PCR techniques for better assessment of risk stratification and guiding the treatment plans or strategies.

CHRONIC MYELOID LEUKEMIA

EPIDEMIOLOGY:

CML constitutes to about 14% of all cases of leukemia, in accordance with the worldwide incidence of CML ranging between 1 to 2 per 1 lakh cases. CML occurs at an increased frequency in adults between 40 and 60 years. Among children, it comprises about 3%, and it accounts for 10% in children of 5–20 years age group [98]. The incidence rate of CML is much lower in India, Netherlands, Sweden and China, whereas it occurs at an increased frequency in Switzerland, USA, Italy, Australia, Germany, and UK ranks [99]. The incidence rate is more common in males than females with the incidence ratio of approximately 3:2 [100].

CLINICAL FEATURES:

Most of the patients remain asymptomatic. Symptoms like fatigue, dyspnea which are the manifestations of anemia may be the presenting symptom. Thrombocytosis is most often noted during the chronic phase of the disease, whereas thrombocytopenia is a common finding in the blast crisis stage. Pallor and massive splenomegaly are the common clinical signs elicited. WBC count is most often >1,00,000 cells/mm³.

PHASES OF CHRONIC MYELOID LEUKEMIA:

- Chronic phase
- Accelerated phase
- Blast crisis

In chronic phase, patients present with increased WBC count of approximately 100,000 cells/mm³ and above. There may be mild reduction in hemoglobin concentration with marked elevation in platelet count. The clinical sign commonly elicited is massive splenomegaly [101]. Complete hematological and cytogenetic remission is achieved by more than 90% of the patients following three months of treatment with imatinib 400mg daily. The transformation to accelerated phase or the blast crisis is characterized by acquisition of few additional chromosomal aberrations and these patients become resistant to imatinib.

According to revised World Health Organization (WHO) classification, the features of accelerated phase is characterized by the presence of >= 1 of the following hematological or cytogenic criteria:

1) Persistent or increasing WBC count of >10 × 10^9/L which is unresponsive to treatment;

2) Persistence of thrombocytosis of >1000 x 10^9/L not responding to treatment.

3) Persistence of thrombocytopenia, with platelet count of less than 100 x 10^9/L not responding to treatment;

4) Occurrence of non random cytogenetic chromosomal abnormality on progression

5) 20% or more basophils in peripheral smear

6) Peripheral blood smear or marrow blasts of 10 to 19% [102].

The WHO criteria for CML-BP include the following:

1) Blast count equal to or more than 20% of the leukocytes in peripheral blood or of the nucleated cells of bone marrow or

2) An extramedullary proliferation of blasts.

Acquisition of the following additional cytogenetic aberrations such as extra Ph, +8, +19 or isochromosome 17q is the reason behind the transformation of chronic to either accelerated or blast phase in most cases of CML [103].

LABORATORY FINDINGS:

The peripheral smear shows a left shift with myelocyte bulge, appearance of blasts and basophils. The blast count and basophils are

increased in the blast crisis phase. The blasts can be myeloblast in 75% of the cases and it is lymphoblasts in 25% of the cases [104].

CHROMOSOMAL ABNORMALITIES:

In 1960, Nowell and Hungerford discovered a deletion in chromosome 22 in patients with CML. In honour of the city it was first identified, the 'shortened' chromosome 22 was called the Philadelphia or Ph chromosome. Cytogenetic studies reveal that the Ph chromosome is present in about 90-95% of CML patients , wherein one chromosome 22 appears to be 'shortened'. The standard t(9;22)(q34.1;q11.2) reciprocal translocation contributes to more than 90% of the Ph chromosome [105]. Variant translocations are present in remaining 5-10% Ph+ CML patients , which may either be complex or simple [106, 107]. Complex involves translocations of chromosomes 9, 22 with one or more other chromosomes; or simple involves translocation of chromosome 22 with a chromosome other than 9. High- resolution banding studies have demonstrated simple and complex variant translocation to cryptically involve translocation of 9q34 to 22q11. In rare cases, a reciprocal translocation between chromosome other than 9 and chromosome 22 appears larger than usual, and the post translocation shortening of the long arms of 22 is inconspicuous. As the 22q- is not detectable by microscopic examination, it has been referred to as a masked Ph

chromosome or masked translocation. The clinical and hematological picture between patients with the standard and variant translocation is almost the same and there are no established significant differences between them. The role of the Ph chromosome in the etiology of CML has not been fully elucidated. Some studies have shown it to be an initial leukemogenic event. In contradictory, some patients with CML had developed the Ph chromosome during the course of the disease. 10-20% of the patients have additional cytogenetic abnormalities even at diagnosis of Ph+ CML, which includes mainly an additional Ph chromosome, trisomy 8 and in males, the loss of the Y chromosome [108]. The presence of these additional abnormalities at diagnosis has been shown to be associated with a poor prognosis. When the disease progression occurs, secondary chromosome aberrations occur in 75-80% of CML patients, which includes most common changes like the gain of chromosome 8, an extra Ph chromosome, and isochromosome for the long arm of chromosome 17 [i(17q)]. Combinations of these changes along with gain of chromosome 19 usually occur later in karyotype evolution. The other least common chromosomal aberrations includes Y, -7, -17, +17, +21 or t(3;21)(q26;q22). Near haploid leukemic stemlines have also been reported [109]. There is a correlation existing between the type of secondary chromosome aberration with the phenotype of the blast crisis. Hyperdiploidy, trisomy 8, 19, 21, and i(17q) are associated with

myeloid blast crisis. An extra Ph is seen in both myeloid and lymphoid blast crisis. Chromosome loss is seldom seen (-7 is the most common) compared to chromosome gain observed patients with blast crisis. Before the blast crisis has been established as a diagnosis in peripheral smear or bone marrow, these aberrations occur 2-4 months earlier. Followup cytogenetic studies has turned out to be an essential tool for monitoring the progression of the disease, especially an impending blast crisis [109, 110]. The Ph chromosome is not present in about 5-10% of CML patients. There are few significant difference in the epidemiology and clinical presentations between the Ph negative (Ph-) patients and Ph+ CML patients which includes the age, sex, survival rates and blood counts. Older age, male sex, shorter survival rate, lower platelet and white blood count points towards a Ph- . The common chromosome aberrations found in Ph- CML patients are trisomy 8 (20%), monosomy 7 (20%), i(17q)(10%), and structural rearrangements of chromosome 3 (>3%) 109. Molecular studies have shown that about 1-2% of Ph- CML patients have *bcr-abl* gene rearrangement. However, although rare, the existence of 'true CML' in Ph- CML patients without *bcr-abl* gene rearrangement have also been found [111].

CHRONIC LYMPHOCYTIC LEUKEMIA

CLL is the most common form of leukemia in adults, among all the leukemias in the western world, but is rare in asia [112]. CLL is a heterogenous disease with most patients having a slow accumulation of neoplastic mature B lymphocytes, thus contributing to the slow progression of the disease, whereas others have a rapid and aggressive course of the disease [113, 114]. The peripheral blood and bone marrow of these patients show persistent absolute increase in morphological mature lymphocytes. The absolute lymphocyte count is elevated. CLL is more frequent in elderly patients. The median age of the patients is 70 years and rare below 50 years of age. The clinical parameters in determining the prognosis include the number of nodes involved, hemoglobin levels and platelet count.

CYTOGENETIC ABNORMALITIES IN CLL:

In contrast to the situation in CML, no specific translocation is found that is characteristic for the majority of the CLL patients. Five major prognostic categories have been defined (Döhner et al., 2000). The most common chromosomal aberrations include deletions in 13q14 in 40-60% of the CLL cases, 11q22-23 deletion in 15-20% and 17p13del in 5-10%. Trisomy 12q has been detected in about 7-30% and about 20% of CLL patients display a normal karyotype [115, 116]. The 13qdel is the most

frequent chromosome alteration in CLL and as the sole aberration it is associated with favorable prognosis [115]. Trisomy12 is found associated with early progression in CLL [117]. The 11qdel is associated with poor survival and extensive lymphadenopathy especially in younger CLL patients [118]. The 17pdel is associated with a very aggressive clinical course [119].

The apparent rarity of chromosomal abnormalities in CLL might be resulting from technical problems related to the use of peripheral blood cultures [120, 121].

KARYOTYPING

Conventional cytogenetics is a cost-effective technique which is simple and allows for an overall view of the genome. Karyotyping is a process of studying the chromosome. It involves arranging the chromosomes in homologus pairs after attaining metaphases, based on the length of chromosome, banding pattern and the centromere position.

HISTORY OF CYTOGENETIC STUDIES:

Cytogenetic studies performed in human cells could be divided broadly into three phases. The first phase was aimed at developing methods to obtain metaphase spreads. Initial culture techniques used skin fibroblasts, and in 1956 Tijo and Levan identified the exact number of

chromosomes in man. Four years later, phytohemagglutinin (PHA) was discovered to have stimulatory effects on T-lymphocytes in the peripheral blood to undergo mitosis. This was a major breakthrough in the cytogenetics, which led to the transformation of cytogenetic analysis from a research tool to a diagnostic tool. In 1960, the first consistent chromosome abnormality to be associated with a malignancy was discovered. It was the Philadelphia chromosome. Giemsa staining was used in the cytogenetic studies from 1956 to 1969, resulting in the uniform staining of chromosomes along their length. But then, the identification of chromosomes was difficult in a situation especially when chromosomes were of similar size and shape. It was later, a nomenclature was accepted at the Denver Conference in 1960 to group chromosomes according to their size and this system was modified at the Chicago Conference held in 1967. The development of chromosome banding techniques from 1969 to 1971 marked the second phase of cancer cytogenetics. The Paris Conference in 1971 laid the convention used for numbering each chromosome and chromosome band. In 1972, the very first consistent translocation involving chromosomes 8 and 21 [t(8;21)(q22;q22)] was identified in AML. It was then also discovered that the Ph chromosome is not due to a deletion but a reciprocal translocation involving chromosomes 9 and 22 [t(9;22)(q34;q11)] [123]. The third stage introduces the use of specific DNA probes to identify genes or

specific chromosome regions. With the introduction of probes labeled with fluorochromes such as painting probes specific for each human chromosome, the specific translocations can be identified. This is known as the fluorescence in situ hybridization (FISH) technique. Comparative genomic hybridization (CGH) is used to detect gain or loss of DNA in a chromosome. All the chromosome abnormalities can be identified by spectral karyotyping (SKY) or multicolour FISH (M-FISH). However this analysis identifies only the chromosome involved but not a specific region of the chromosome.

KARYOTYPING PROCEDURE

The traditional karyotyping requires actively dividing cells like bone marrow, lymph nodes. If peripheral blood is used, it requires the addition of mitotic agents like PHA for the stimulation of cells for mitosis. The choice of specimen in hematological malignancies is generally bone marrow aspirate. However, peripheral blood can be used.

a) Specimen Collection:

Bone marrow specimen must be collected into sterile tubes containing preservative-free, sodium heparin. The first few milliliters of the bone marrow tap contain the highest proportion of cells and are the best sample for cytogenetics studies. Blood dilutes the bone marrow

sample in later taps and reduced the number of actively dividing cells. The success of bone marrow culture depends on the number of dividing cells. Transport specimen immediately at room temperature to the laboratory. If delay is unavoidable store the specimen at 4 0C and transport in a vacuum flask packed with ice. Bone marrow specimens should be processed immediately upon receipt to avoid cell death. It is better if the bone marrow specimens reaches the laboratory on the same day of collection, or the latest the next day.

b) Culture media:

Specimens for chromosome studies are grown in aqueous growth media such as RPMI 1640, MEM. Culture media are balanced salt solutions containing salts, glucose, and buffering system to maintain the proper pH. Culture media that are obtained commercially are usually incomplete. They have to be supplemented with L-glutamine, fetal bovine serum (FBS) and antibiotics. For bone marrow culture, PHA is not added. L-Glutamine is an amino acid essential for cell growth. It is unstable and breaks down on storage to D-glutamine. L-glutamine must be stored frozen to retain its stability. It is best added to the culture medium just prior to its use.

Serum is essential for good cell growth. About 10-30% FBS is usually added to the culture medium. Penicillin/streptomycin, kanamycin,

and gentamicin are bacterial inhibitors commonly used in tissue culture media to retard the growth of microorganisms. Mitotic stimulant such as PHA is not used for bone marrow culture. However, B cell mitogens (e.g. Epstein Barr virus, pokeweed) can be used to stimulate B lymphocytes

of B-cell leukemia and lymphoma. Blood and bone marrow samples which consist of free floating cells can be cultured in sterile centrifuge tubes or tissue flasks.

c) Preparation of specimen for culture:

Whole blood or bone marrow can be added directly to the culture medium or the white blood cells can be separated from the other blood elements and used to inoculate the culture medium. Separation of the white cells can be accomplished by centrifuging the sample or allowing it to rest undisturbed until the blood settles into three distinct layers. The lowest layer consists of the heavier red blood cells, the top layer consists of plasma, and the narrow middle layer, the buffy coat consists of the desired white cells. Remove the buffy coat and resuspend in culture media. Bone marrow cells are cultured at 37°C in an incubator. They can be harvested directly, without any time in culture, or a 24-hr to 48-hr culture time to increase mitotic index. Longer culture periods are generally not advisable because the abnormal cancer cells may be lost over time or be diluted out by normal precursor cells that may be present.

An overnignt culture for bone marrow specimens is being done in most of the laboratories.

d) Cell harvest:

After the cell cultures have been grown for the appropriate period of time and there is sufficient number of dividing cells, the cells are harvested. Harvesting involves collection of dividing cells at metaphase, hypotonic treatment, fixation, the placement of chromosomes on glass slides, so that they may be stained and microscopically examined. A mitotic inhibitor, colcemid which is an anologue of colchicine is usually used to obtain an adequate number of cells at metaphase. Colcemid binds to the protein tubulin, obstructing the formation of mitotic spindles or destroying those already present. This prevents the separation of the sister chromatids in anaphase, thus collecting the cells at metaphase. Exposure time to colcemid is important. A longer exposure results in more metaphases being collected, but they will be shorter because chromsomes condense as they progress through metaphase. Longer chromosomes are generally preferred for cytogenetic studies. Hypotonic solution is added to the cells after exposure to colcemid. Water enters the cell by osmosis, thus causing the cells to swell. This is critical for adequate spreading of the chromosomes on the slide. Timing is critical. Too long an exposure will cause the cells to burst and too short an exposure will cause

clumping of chromsome spreads. Examples of hypotonic solutions are 0.075M potassium chloride (KCl), 0.8% sodium citrate, diluted balanced salt solutions, dilute serum, and mixtures of KCl and sodium citrate. Morphology of the chromosomes is affected by the type of hypotonic solution used. Fixative (modified Carnoy's solution) containing three parts of absolute methanol to one part glacial acetic acid is used to stop the action of the hypotonic solution and to fix the cells in the swollen stage. The fixative also lyses any red blood cells present in the sample. The fixative must be prepared fresh before use because it absorbs water from the atmosphere. The fixed cell suspension are dropped onto glass slides. The concentration of the cell suspension can be adjusted to achieve optimal results. A good slide preparation has sufficient number of metaphases that are not crowded on the slide, metaphases that are well spread with minimal overlapping of the chromosomes, and no visible cytoplasm. Increased temperature and humidity enhance chromosome spreading, whereas cooler temperature and lower humidity decrease it. Longer exposure to hypotonic treatment makes the cell more fragile and increases spreading, but an inadequate exposure can result in cells that are difficult to burst. Variables in slide preparation include the following:

- height from which the cells are dropped
- the use of wet or dry slides

- the use of cold, room temperature, or warm slides
- the use of steam air –or flame-drying the slides

The slides are 'aged' overnight at 60°C or for 1 hour at 90°C to enhance the chromosome banding. Chromosomes can also be 'aged' by brief exposure to UV light 123.

e) Chromosome banding and staining:

Chromosome staining and banding techniques are divided into two broad categories:

(i) Those that produce specific alternating bands along the length of each entire chromosome

(ii) those that stain only a specific region of some or all chromosomes.

Techniques that create bands along the length of the chromosome include Gbanding (Giemsa banding), Q-banding (Quinacrine banding), and R-banding (Reverse banding). This property enables the positive identification of the individual chromosome pairs and permits the characterization of structural abnormalities. Banding resolution is an estimate of the number of light and dark bands in a haploid set. The minimum estimate is about 400 bands. Well-banded, moderately high

resolution metaphases are usually in the 500-550 band range, and prometaphase cells can achieve a resolution of 850 or more bands.

(i) G-Banding:

G-banding is the most widely used routine banding method. GTG banding (G bands produced with trypsin and Giemsa) is one of the several G-banded techniques. The 'aged' slides are treated with the enzyme trypsin and stained with Giemsa. Besides Giemsa stain, Wright or Leishman stain can be used. A series of light and dark bands are produced which enables the positive identification of each chromosome. The dark bands are A-T rich, late replicating, heterochromatic regons of the chromosomes, whereas the light bands are G-C rich, early replicating, euchromatic regions. The G-light bands represent the most active regions of the chromosomes compared to the G-dark bands which contain relatively few active genes.

(ii) Q-banding:

Q-banding is a fluorescent technique. Q-banding is similar to G-banding pattern with some notable exceptions. The large polymorphic pericentromeric regions of chromosomes 1 and 16, and the distal long arm of the Y chromosome fluoresce brightly. The distal long arm of the Y chromosome is the most fluorescent site in the human genome. Q-

banding is useful to confirm the presence of Y material. The disadvantage of fluorescent stains is that it is not permanent, require the use of expensive fluorescence microscopes and a darkened room.

(iii) R-banding:

R-banding techniques produce a banding pattern that is opposite or reverse of G banding pattern. There are fluorescent and non fluorescent methods. Many human chromosomes have euchromatic terminal ends that can be difficult to vitualise with standard G-band techniques because the pale telomeres may fade into the background. R-banding is useful for evaluation of these telomeres.

(iv) Other banding techniques:

Techniques that stain selective chromosome regions include C-banding (Constitutive heterochromatin banding), T-banding (Telomere banding), Cd staining (Centromeric dot or kinetochore staining), G-11 banding (Giemsa at pH 11), NOR staining (Silver staining for nucleolar organizer regions), and DAPI/DA staining (4,6-Diamino-Phenole-Indole/Distamycin A).

- C-banding selectively stain the constitutive hetrochromatin around the centromeres, the areas of inherited polymorphisms present on chromosomes 1,9, 16, and the distal long arm of the Y chromosome. It is

useful for determining the presence of dicentric and pseudodicentric chromosomes, and also for studying marker chromosomes.

- T-banding results in only the terminal ends or telomeres of the chromosomes being stained.
- Cd staining produces a pair of dots at each centromere, one on each chromatid. Only the active or funtional centromeres will be stained. Cd staining can be used to differentiate functional from nonfunctional centromeres, to study Robertsonian translocations, ring chromosomes and marker chromosomes.
- G-11 banding specifically stains the heterochromatin regions of chromosomes 1, 9, 16, the distal Yq, and the satellites of the acrocentric chromosomes. It is used to differentiate between human and rodent chromosomes in hybrid cells.
- Nor staining selectively stains the NORs located on the satellite stalks of the acrocentric chromosomes. The silver stains only the active ribosomal RNA genes. Nor staining is useful for the identification of marker chromosomes and rearrangements or polymorphisms involving the acrocentric chromosomes.
- DAPI/DA staining combines DAPI, a fluorescent dye, with distamycin A, a non fluorescent antibiotic. Both form stable bonds to similar A-T rich, double stranded regions of DNA. Used together, DAPI/DA fluoresces certain A-T rich areas of

constitutive heterochromatin in the C band regions of chromosomes 1,9,16, and distal Yq, and the short arm of chromosome 15. DAPI/DA is used to identify rearrangements of chromosomes 15; to confirm variations in the polymorphic regions of chromosomes 1,9, 16, and distal Yq, and to study marker chromosomes with satellites.

Culture Failure:

There are five reasons for culture failure. These include:

1) Inadequate Banding

2) Too few metaphases were available for analysis, which includes cases where no analyzable metaphases were obtained

3) The local cytogenetics laboratory or the central reviewer deemed the specimen to be inadequate, which includes cases of pre-analytical errors where wrong anticoagulant used, insufficient mix with even the correct anticoagulant leading to clotting, too much time spent in transit/extreme temperature exposure during transportation, labeling error, hemolysed specimen, or low cell count

4) Processing of the sample was inadequate which usually refers to incorrect tissue culture conditions or only one culture set up

5) The sample did not contain any living cells. This could be due to delay in sample transport or the specimen was not kept at 4°C and transported in ice, if the delay is unavoidable.

6) The laboratory suffers from a catastrophic equipment failure.

7) Reagent failure.

Automation in the cytogenetics laboratory:

Cytogenetic techniques are labour intensive. Instruments such as robotic harvesters, environmentally controlled drying chambers, and computerized imaging systems have been developed to assist the chromosome laboratory in sample preparation and chromosome analysis (Gersen et al, 1999). Some cytogenetic laboratories use all of these devices, some use one or two, and some do not use any. The Cytogenetics Laboratory at the IMR started to use the computerized imaging system in January1998. Except for the imaging system, the other techniques for cytogenetic studies at the IMR are performed manually.

a) Robotic harvesters:

Robotic harvesters can perform harvesting of mitotic cells for cytogenetic analysis after the addition of colcemid. The robotic machine can perform aspiration, dispensing of hypotonic solution, and as well as fixative. The incubation times for each step are programmed into a computer that controls the robot.

b) Drying chambers:

Spreading of chromosomes is achieved by placing one or more drops of this suspension on a number of slides. Chromosome spreading is controlled by the height from which the suspension is dropped, the temperature and the condition of the spread, humidity and air flow of the surrounding. As the cell suspension dropped on the slides dries, the fixative pulls the cell membrane across the slide, allowing the chromosomes of the cells to spread. Conditioned controlled chambers where air flow, humidity and temperature can be controlled for routine slide preparation are available commercially.

c) Computerized imaging:

The traditional method of karyotyping involves photomicroscopy after the location of suitable metaphases using an England finder. A camera is attached on top of the microscope to take photographs of the

metaphase spread. The film and photographs is processed in a dark room. The metaphase spreads are cut and paired. Karyotyping by this procedure is time consuming.

The above process can now be performed using a computerized imaging (fully or semi automatic). An image acquisition subsystem can be used to capture the metaphase spreads. The subsystem consists of a microscope camera adapter, a charged couple device (CCD), a frame grabber and an image capture software. Dedicated cytogenetic imaging software can perform karyotyping (either automatically or semi-automatically), banding analysis and ideogram display are all now commercially available. Optional components such as metaphase finding, FISH analysis (including M-FISH), CGH analysis can be included. Metaphase spreads are captured in the digital form by the Cytovision satellite capture station. The image is then transferred to the Cytovision workstation where karyotyping is performed. Karyotyping is performed semi-automatically. The geneticists have to check the karyotypes manually to ensure that the chromosomes are correctly paired. A laser printer, Lexmark then produces a hardcopy of the metaphase spread and karyotype. The images consisting of metaphase spreads and karyotypes are stored as digital files on the optical disks.

MATERIALS AND METHODS

A total of 30 cases are taken for the study. This is a prospective study conducted for a period of 1.5 years, with the study centre being Institute of Pathology, Rajiv Gandhi Government General Hospital. All the cases are diagnosed to be either AML, ALL, CML or CLL based on the peripheral smear findings, in correlation with the CBC values.

INCLUSION CRITERIA:

All cases of newly diagnosed Acute and Chronic leukemia based on clinical and laboratory parameters.

EXCLUSION CRITERIA:

- Patients with a low total WBC count in leukemia
- Patients diagnosed with leukemia but with low blast count in acute leukemia
- Leukemic patients on treatment
- CML transforming into AML
- MDS transforming into AML

Peripheral blood from all the 30 cases were run in a 5 part hematology analyzer and confirmed and verified to have high count with the peripheral smear. The morphology of the immature blast cells were

studied and were classified as myeloid or lymphoid in case of acute leukemia. Further subtyping was also made based on the nuclear and cytoplasmic characteristics and the presence or absence of nucleoli in the blast cells.

MATERIAL FOR CYTOGENETIC STUDY:

Peripheral blood sample from the leukemic patients was used for the study. The study was conducted at Rajiv Gandhi Government General hospital in the Institute of Pathology.

METHODOLOGY:

For cytogenetic studies all chemicals, culture tubes, containers and glassware for cell culture are kept sterile.

Sample Collection:

The peripheral blood of 2ml was collected by venipuncture from the patients, after obtaining consent from the patient. The sample is transferred to heparin tube (green top tube). The sample is transferred to the laboratory immediately with a special request form (Annexure I). In case, if the sample cannot be transported immediately to the laboratory, it is stored in the refrigerator at 4-8°c and processed on the same day evening.

Sample assessment:

Once the sample enters the laboratory, the tube top, name on the tube and the sample quantity are assessed. The name on the tube is matched with the name on the request form. Preanalytical errors are avoided in this manner.

Cell density calculation:

Before setting up the culture, the cell density of the sample is assessed in the following manner:

1) When samples reach the Genetics Laboratory, centrifuge the sample at 900 rpm for 10 minutes

2) Discard the supernatant

3) Mix the cell pellet

4) Aliquot 1.9ml 2% acetic acid into a tube containing 0.1ml of cell pellet.

5) Place one drop of suspension to one side of the modified neubauer chamber.

6) Count all cells in the 16 squares of the modified neubauer chamber using a microscope.

7) The amount of cell density to be added to 5ml of culture media is calculated as per the following formula:

(7.5/X) x100= µL of sample

For bone marrow, count WBCs manually in Neubauer chamber. Add 20 µL of peripheral blood to 280µL of glacial acetic acid and charge the neubauer chamber. Count the number of cells in 4 large squares.

8) Record the cell density count and amount of sample required per culture.

Culture setting:

1) Add 10 ml of culture media to 150-1000µL in culture tube, using micropipettes

2) Add 40µL of colcemid to the tube.

3) Incubate overnight at 37°C in incubator.

Harvesting:

Harvesting is done on day 2.

1) Transfer the contents to conical centrifuge tube
2) Centrifuge the samples at 1000 rpm for 10 minutes. Discard the supernatant.

3) Add pre-warmed KCl to make up to 8 ml and incubate at 37°c for 20 minutes
4) Centrifuge the sample at 1000 rpm for 10 minutes. Discard the supernatant
5) Add ice cold fixative to make upto 8 ml.
6) Caution should be made while adding the fixative. The felcon tube is placed in a vortex mixture and only then, the fixative is added. If not, there are increased chances of clot formation.
7) Centrifuge the sample at 1000 rpm for 10 minutes. Discard the supernatant
8) Repeat steps 5 & 6 until a clear white pellet is formed (2-3 times). Keep a small amount of fixative depending on the pellet size.
9) Keep the pellet in refrigerator at 4°c overnight

Slide making:

1) Gently dilute and resuspend pellet with additional fixative to give a slightly cloudy suspension.
2) Assess cell suspension using a microscope.
3) If the preparation is too dense, remake slide using a diluted cell suspension.

4) If suspension is too dilute, spin down again and resuspend in less fixative. If the spreads are clumped or in cell membrane, wash with fixative one more time and redrop the slide.
5) Spot the sample to a clean glass slide from a height of 20cm and place it in slide warmer at 40°c for 2-3 minutes until dry
6) Make 8-10 slides per case
7) Store excess cell suspension in fixatives at –20oC. To remake slides, spin down suspension and change fixative once before making slides.
8) Age slides in the oven (56°C) for at least 3 days.

G-Banding:

1) Prepare fresh trypsin solution 0.125% with pH7.3 phosphate buffered saline (PBS).
2) Prepare four coplin jars each containing the following solutions:
   - Trypsin solution
   - PBS I
   - PBS II
3) Treat slides for 10-20 seconds by slowly agitating in trypsin solution kept at 37°c
4) Rinse in 2 series of 1x Phosphate Buffered Saline (PBS)
5) Stain in Giemsa for 3-5 minutes

6) Rinse in 2 series of distilled water and air dry

7) Mount a coverslip on the slide and check under microscope for chromosome bands. If under banded increase the trypsin treatment timing. If it is overbanded decrease the trypsin treatment or the PBS timing, whichever is appropriate.

8) Screen for good quality metaphase spreads.

Screening of metaphase spread:

1) Each banded slide is screened for well banded metaphase spreads using a bright field microscope.

2) Screen and record the position for at least 20 good metaphase spreads.

Capturing of metaphase spreads:

1) Capture at least 20 metaphase spreads using a satellite capture station was done.

2) The images are transferred to an image analyser.

Karyotyping of chromosome spreads:

1) Load the particulars of the patients in the image analyser

2) Karyotyping is performed using an image analyser, which automatically matches and separates the chromosome groups based on the ideogram, already programmed.

3) Human cytogenetic nomenclature is reported based on ISCN (1995).

OBSERVATIONS AND RESULTS

A total of 30 cases are included in the study. The cases are detected with a high count in CBC analyzer and confirmed with peripheral smears stained with leishman stain and special stains (MPO, PAS) and diagnosis was made. The karyotyping results of the cases are as follows: 30 newly diagnosed leukemic patients, not on any treatment were included in the study, as the treatment may cause alteration in the karyotype and may interfere with the culture. The clinical parameters, blood count parameters, peripheral smear findings are correlated with the karyotype.

S NO	AGE	SEX	DIAGNOSIS	KARYOTYPE
1	45	M	AML	46xy
2	45	M	CML	46xy, t(9;22)(q34;q11.2)
3	45	M	CML	46xy, t(9;22)(q34;q11.2)
4	27	M	AML	46xy, t(9;22)(q34;q11.2)
5	28	F	Biphenotypic leukemia	45xx,Del(6)(q21q25)
6	32	M	CML chronic phase	46xy, t(9;22)(q34;q11.2)
7	70	F	AML	46xx
8	60	M	CML	46xy, t(9;22)(q34;q11.2)
9	20	F	AML	46xx
10	64	M	CML stable phase	46xy, t(9;22)(q34;q11.2)
11	21	F	ALL	47xx,+4, add(12)(q24.1)-14, +15
12	55	F	CLL	46xx
13	65	F	CML	46xy, t(9;22)(q34;q11.2)
14	52	F	AML	46xy,t (15;17)
15	35	M	ALL	45xx,Del (20q)
16	54	M	CML	46xy, t(9;22)(q34;q11.2)
17	48	F	CML	46xy, t(9;22)(q34;q11.2)
18	34	M	CML	46xy, t(9;22)(q34;q11.2)
19	42	M	AML	46xy,t(15;17)
20	58	F	CML	46xx, t(9;22)(q34;q11.2)
21	56	F	CML	46xx, t(9;22)(q34;q11.2)
22	40	F	AML	46xx,t(8;21)
23	48	M	AML	46xy, t(9;22)(q34;q11.2)
24	38	F	CML	46xx, t(9;22)(q34;q11.2)
25	32	M	CML	46xy,t(3,6,9,22)(p13;p21;q34;q11.2)
26	23	M	AML	46xy,t(8;21)
27	14	F	ALL	46xx,t(12;21)
28	16	M	ALL	46xy,t(9;22)(q34;q11.2)
29	46	F	AML	46xx
30	56	M	CLL	46xy

13 cases had CML morphology on smear, of which 12 cases had the BCR-ABL translocation and 1 case was Ph negative. 10 cases had the preponderance of myeloblasts on smear and diagnosed as AML, of which

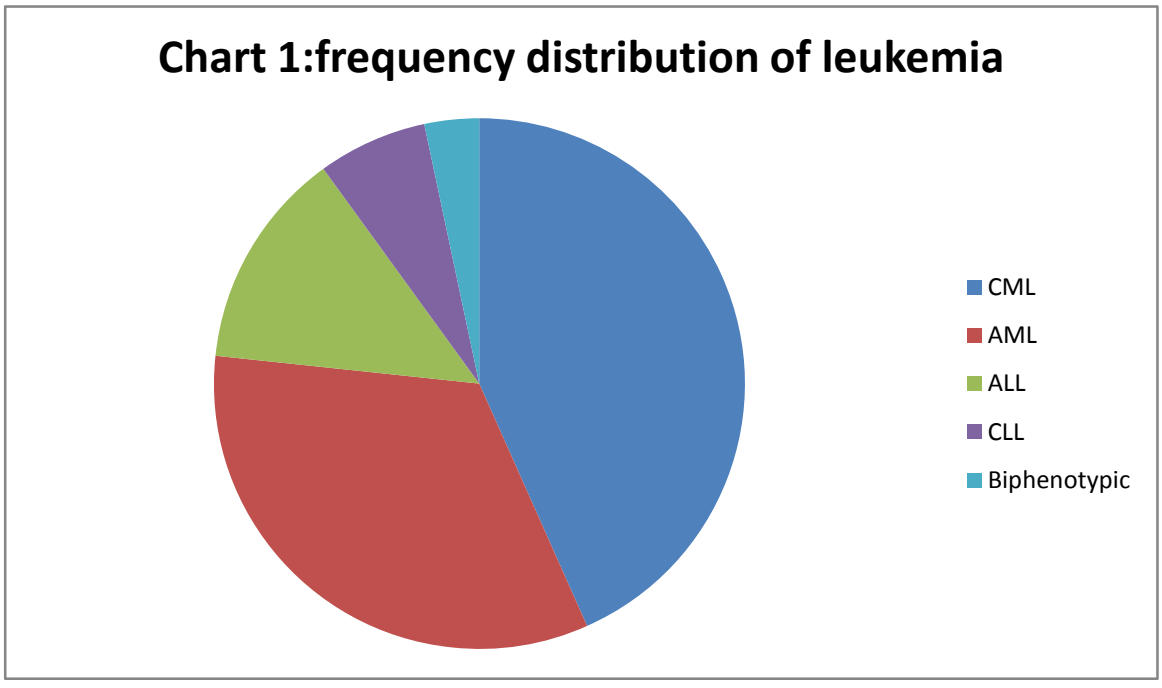
4 cases had normal karyotype, 2 cases had t(15;17), 2 cases had t(8;21), 1 case had t(4;11) and 1 case had BCR-ABL translocation. 4 cases had the preponderance of lymphoblasts in peripheral smear and diagnosed as ALL. All 4 cases had different karyotypes, which includes: 47xx,+4add(12q24.1)-14+15 and 20q-, t(12;21) and BCR-ABL translocation. There were 2 cases of CLL with normal karyotype and 1 case of biphenotypic leukemia, which had 2 different populations of blasts on peripheral smear showed the karyotype of del(6)(q21q25).

Frequency of distribution of leukemias:

Out of the total 30 cases, 43.33% (13 cases) were CML, 33.33% (10 cases) were AML, 13.33% (4 cases) were ALL, 6.66% (2 cases) were CLL and 3.33% (1 case) was biphenotypic leukemia. The immunophenotyping of the 1 case was planned to confirm the biphenotypic leukemia, but was withdrawn due to financial constraints.

Table 1 Frequency of distribution of leukemias

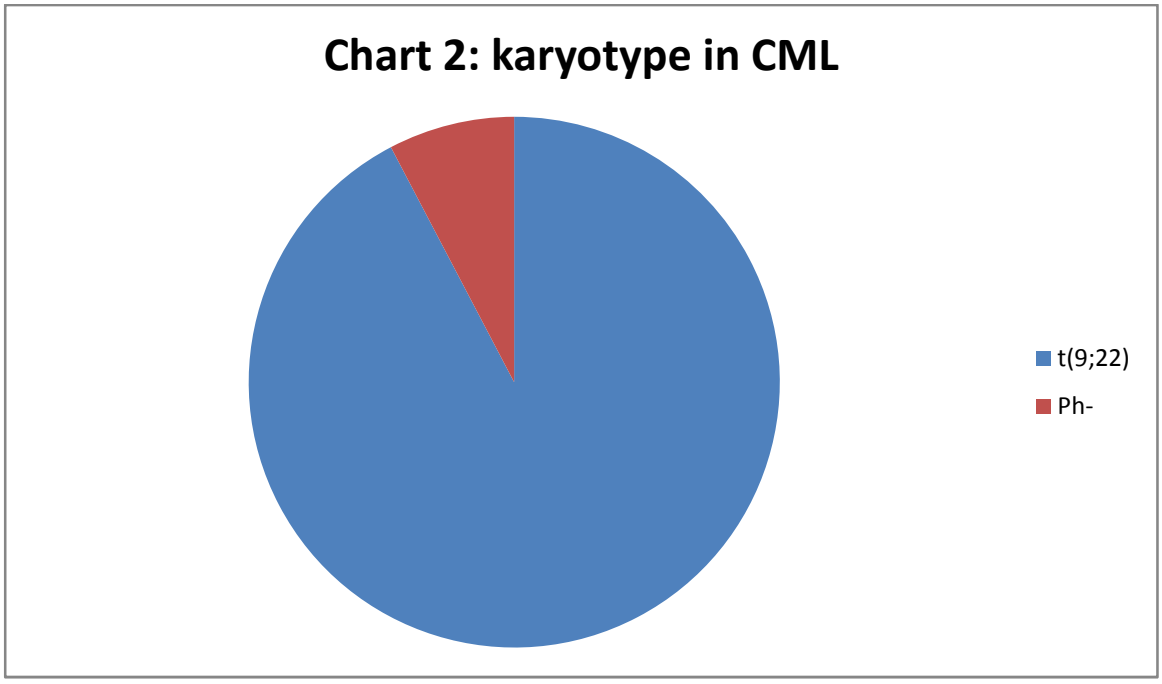
S NO	DIAGNOSIS	FREQUENCY	%
1	CML	13	43.33%
2	AML	10	33.33%
3	ALL	4	13.33%
4	CLL	2	6.66%
5	Biphenotypic leukemia	1	3.33%



CML was the most common leukemia observed in this study, followed by AML, ALL, CLL and biphenotypic leukemia in decreasing order of frequencies.

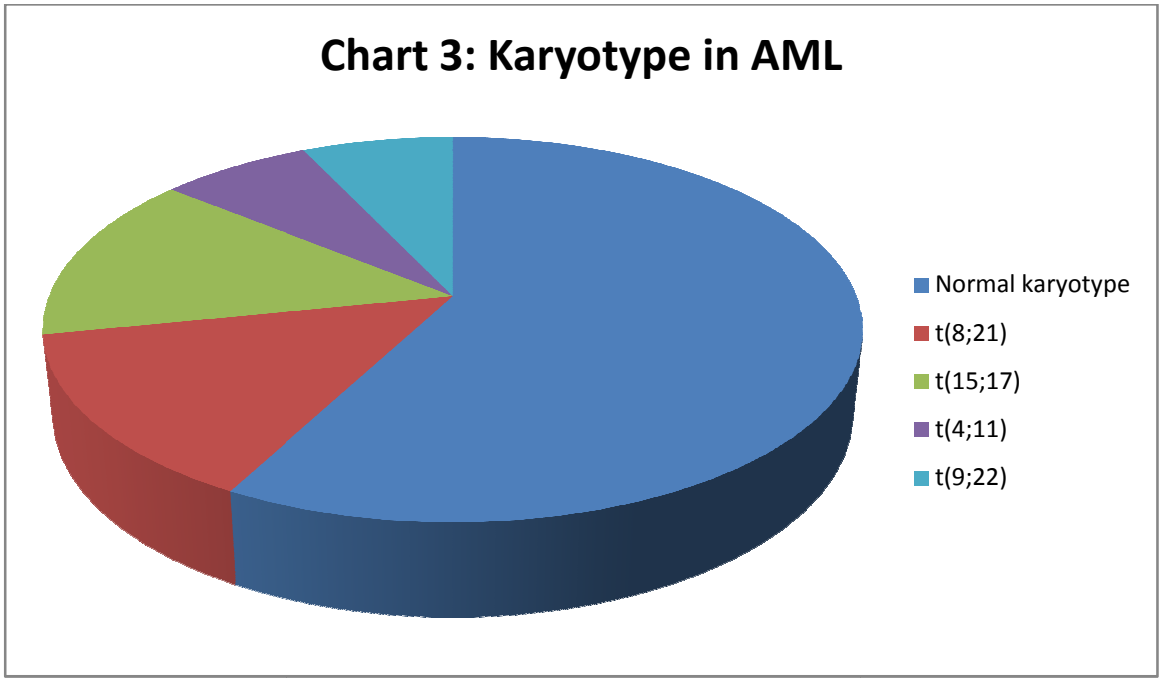
Distribution of karyotype in CML:

Of the 13 cases observed, 92.30% (12 cases) had t(9;22),whereas 7.69% (1 case) had Ph-ve.

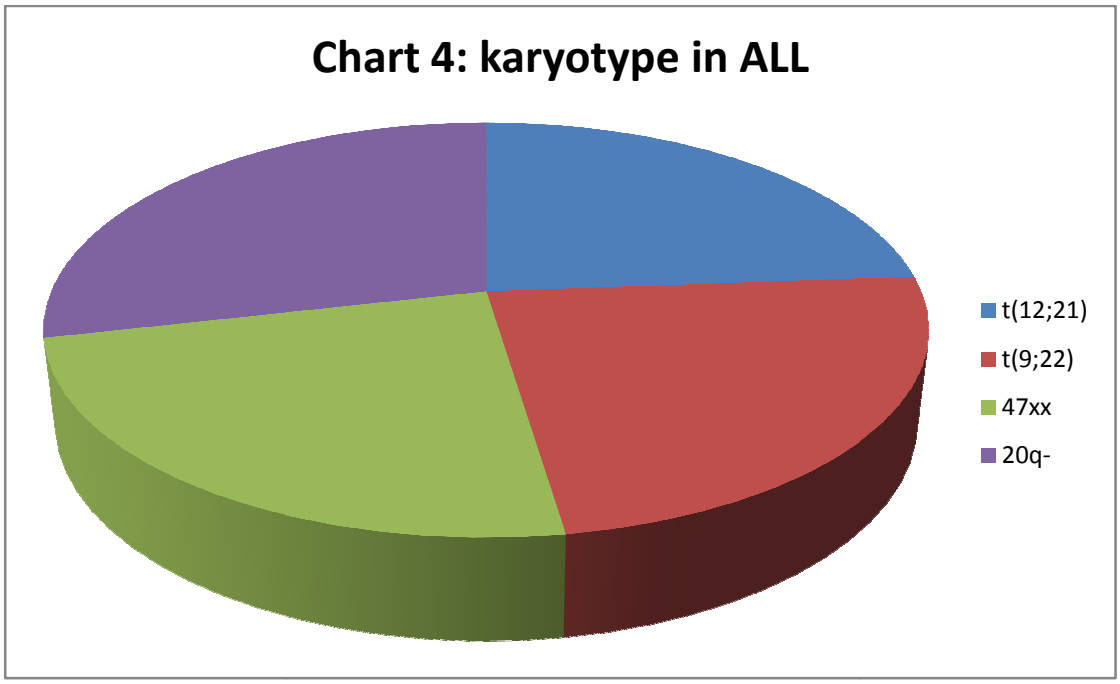


Of the 10 cases in AML, majority of 40%(4 cases) had a normal karyotype, 20% (2 cases) had t(8;21), 20%(2 cases) had t(15;17), 10%(1 case) had t(4;11) and 10%(1 case) had t(9;22).

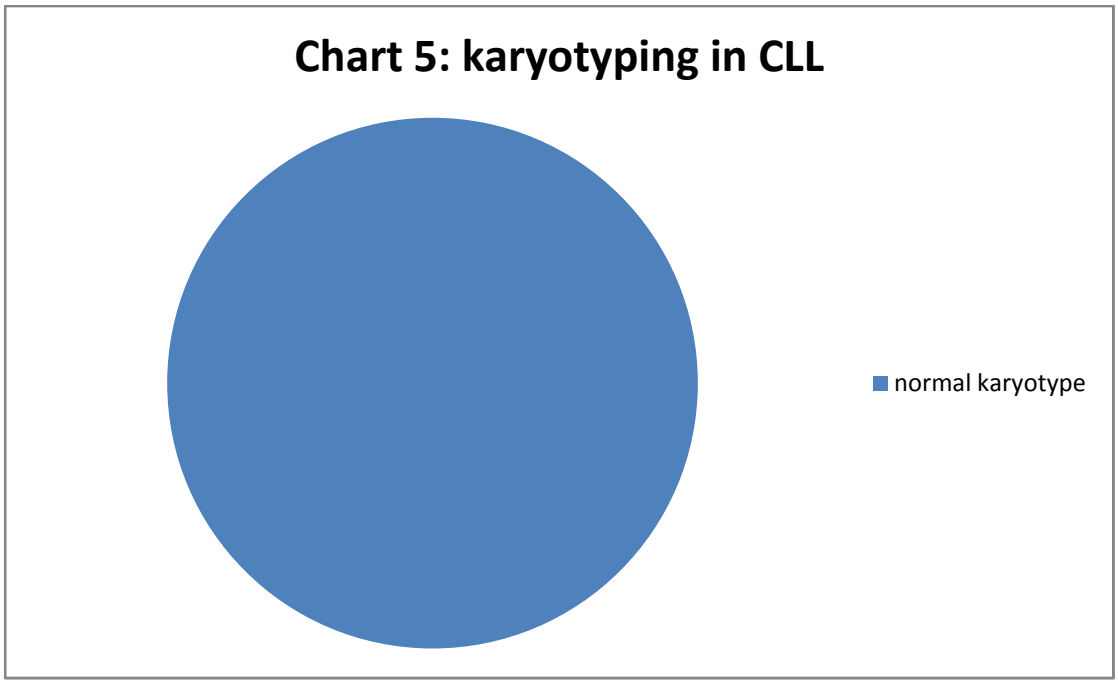
The following chart illustrates the distribution of various karyotypes in AML.



All the 4 patients of ALL had different karyotypes, each contributing to 25%. These include t(12;21), t(9;22), 47xx, 20q-. The following chart indicates the distribution of different karyotypes in ALL patients.



2 cases of CLL were observed in the study, both having normal karyotype(100%). The following chart illustrates the distribution of karyotype in CLL patients.



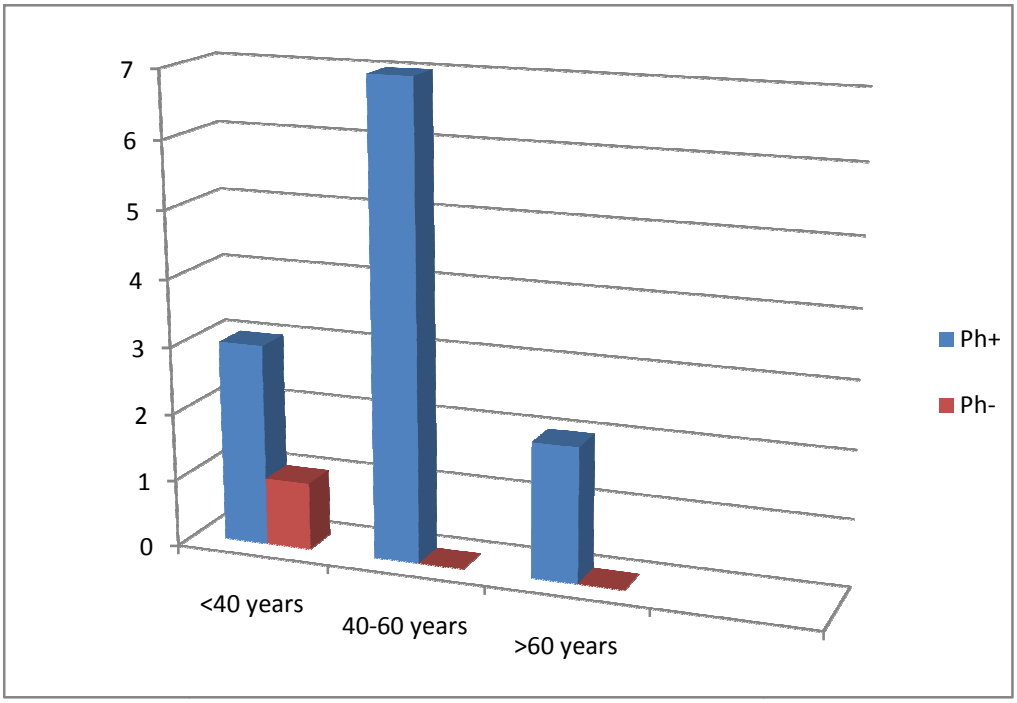
Age distribution:

Among the CML patients, 53.84% (7 cases) were between 40-60 years of age, 30.76% (4 cases) presented in less than 40 years of age, 15.38% (2 cases) were more than 60 years. The age distribution of CML patients with the karyotype is depicted in the table.

Table 2: Age distribution of karyotype in CML

Age range	Ph+	Ph-	% total in each group
<40 years	3 (23.07%)	1 (7.69%)	30.76%
40-60 years	7 (53.84%)	-	53.84%
>60 years	2 (15.38%)	-	15.38%

Chart 6: Age distribution of karyotype in CML

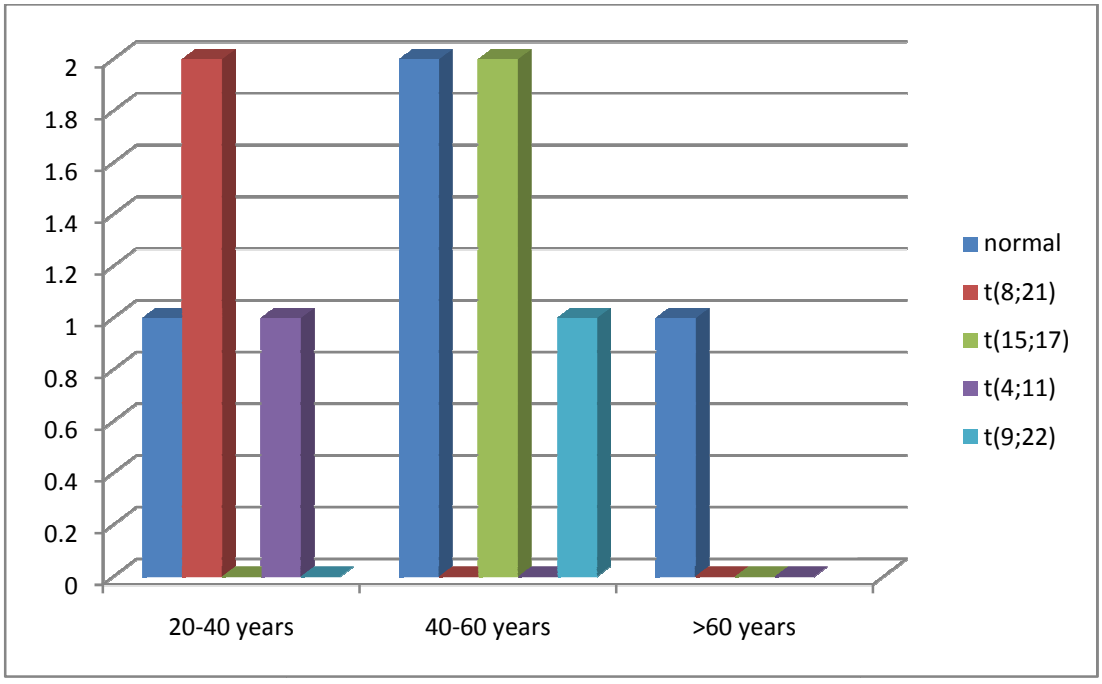


Among AML patients, of the total 10 cases, 40% (4 cases) were between the age group of 20-40 years. 50% cases were between the age group of 40-60 years. Only one case (10%) contributed to patients more than 60 years.

Table 3: Age distribution of karyotype in AML

Age range	Normal	t(8;21)	t(15;17)	t(4;11)	t(9;22)	%
20-40 yrs	1(10%)	2(20%)	0	1(10%)	0	40%
40-60 yrs	2(20%)	0	2(20%)	0	1(10%)	50%
>60 yrs	1(10%)	0	0	0	0	10%

Chart 7: Age distribution of karyotype in AML

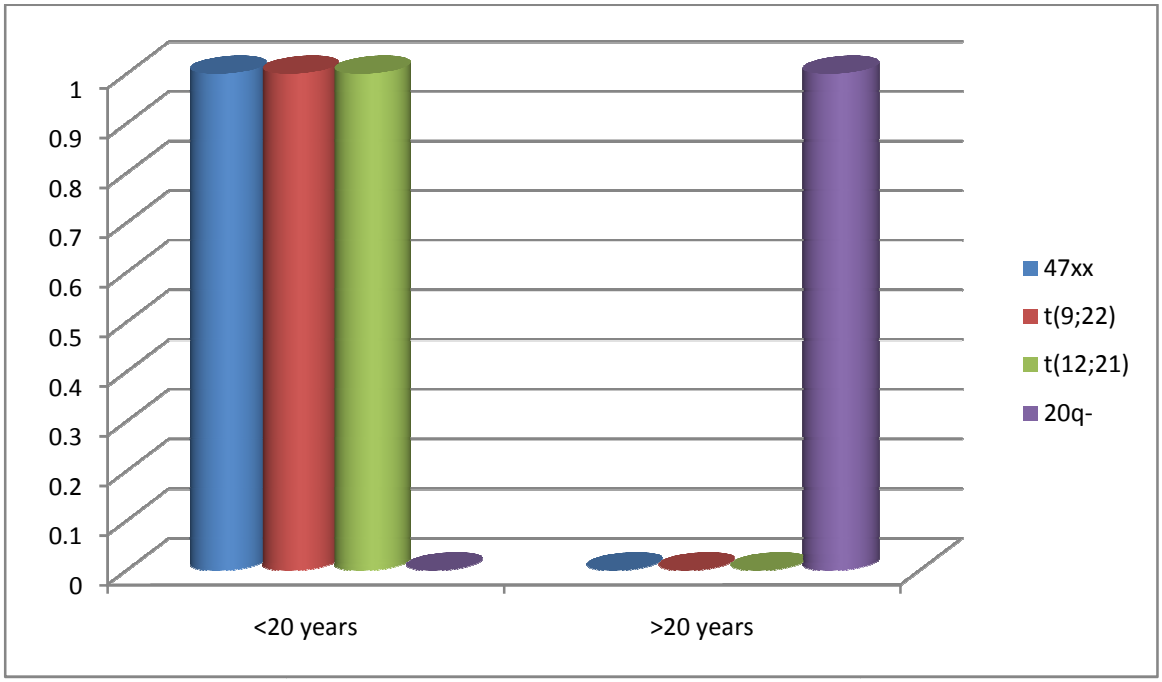


Out of the 4 cases of ALL, 75% (3 cases) are distributed in less than 20 years of age, whereas 25% (1 case) was noted in more than 20 years of age.

Table 4: Age distribution of karyotype in ALL

Age range	47xx	t(9;22)	t(12;21)	20q-	%
<20 years	1(25%)	1(25%)	1(25%)	0	75%
>20 years	0	0	0	1(25%)	25%

Chart 8: Age distribution of karyotype in ALL

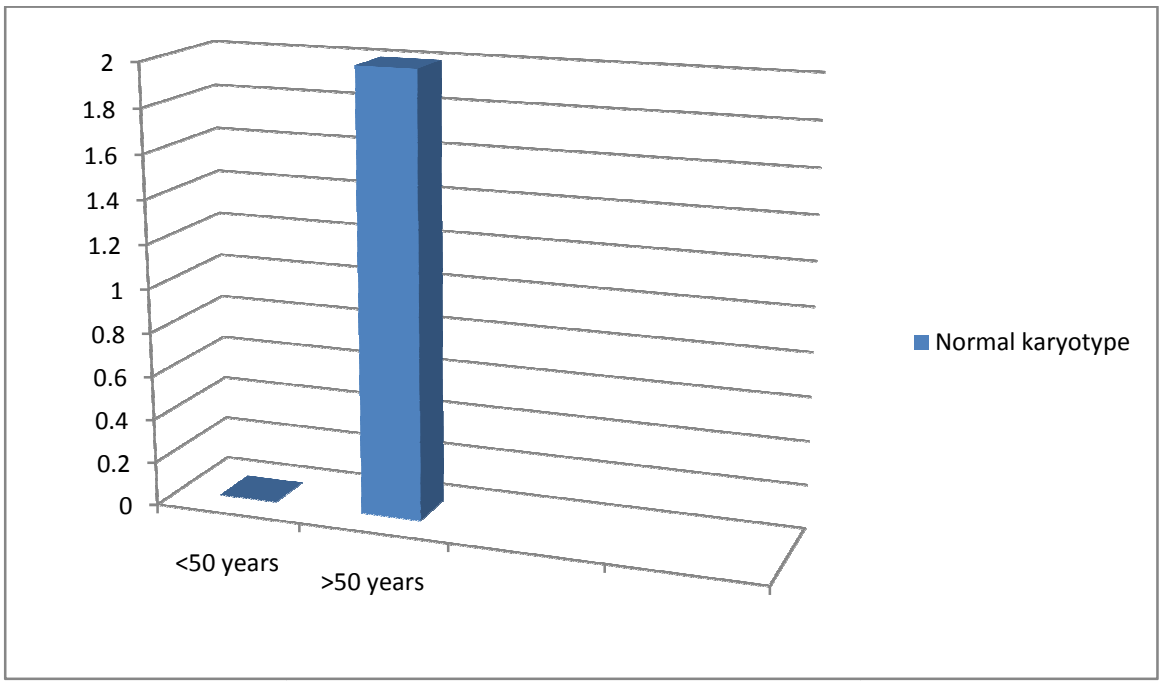


2 cases of CLL were present in age group >50 years of age.

Table 5: Age distribution of karyotype in CLL

Age range	Normal	%
<50 years	0	0%
>50 years	2	100%

Chart 9: Age distribution of karyotype in CLL



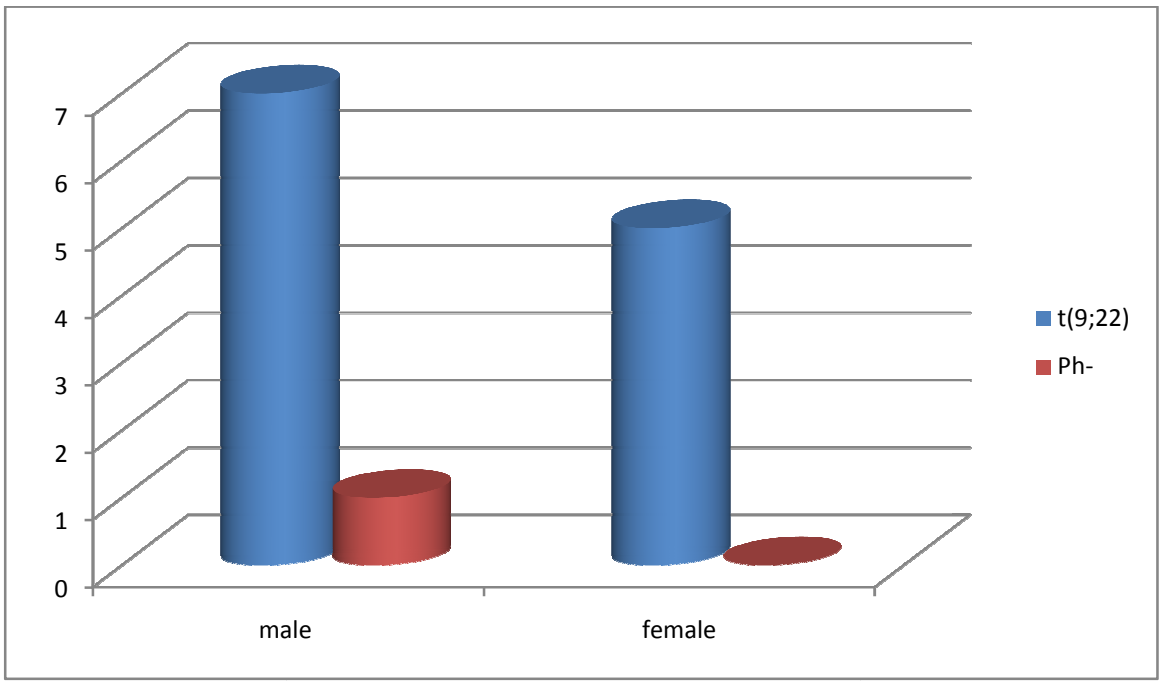
Sex distribution:

Among the 13 cases of CML, 76.92% (10 cases) were present in males, of which 69.23%(9 cases) had Philadelphia chromosome, whereas 7.69% (1 case) was Ph negative. 23.07% (3 cases) was had Philadelphia chromosome.

Table 6: Sex distribution of karyotype in CML

Sex	t(9;22)	Ph-	%
Male	9(69.23%)	1(7.69%)	76.92%
Female	3(23.07%)	0	23.07%

Chart 10: Sex distribution of karyotype in CML

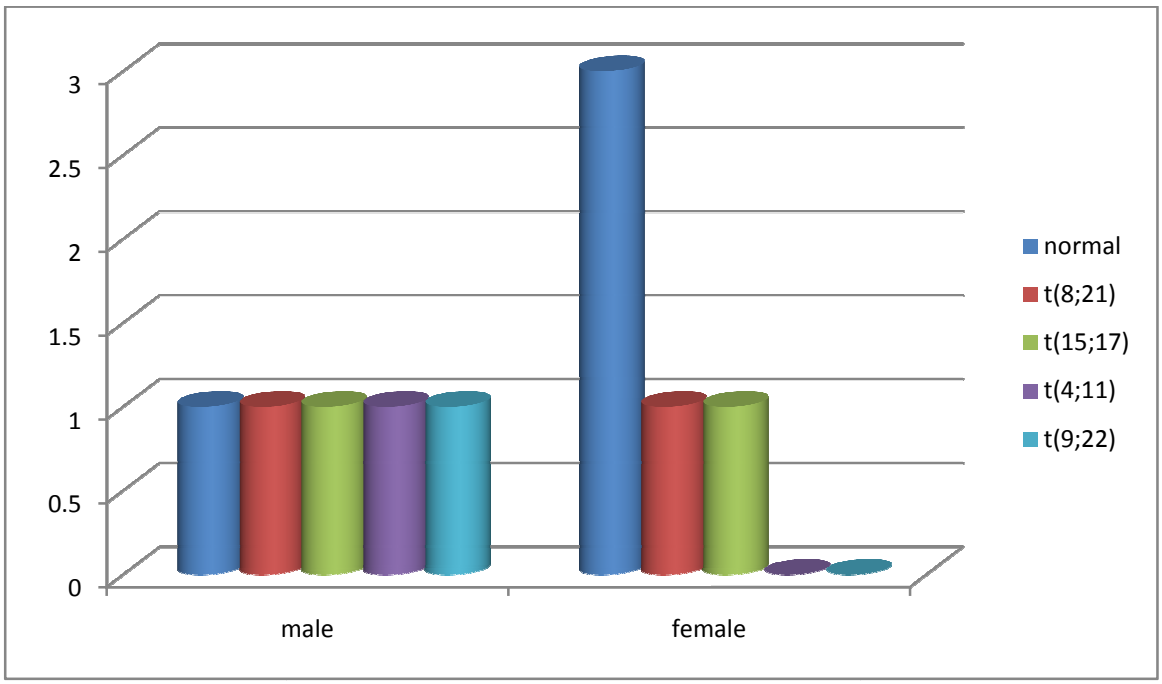


Among the 10 cases of AML, 5 cases occurred among both males and females. The following table indicates the distribution of various karyotypes observed in AML among both sexes.

Table 7: Sex distribution of karyotype in AML

Sex	Normal	t(8;21)	t(15;17)	t(4;11)	t(9;22)	%
Male	1(10%)	1(10%)	1(10%)	1(10%)	1(10%)	50%
Female	3(30%)	1(10%)	1(10%)	0	0	50%

Chart 11: Sex distribution of karyotype in AML

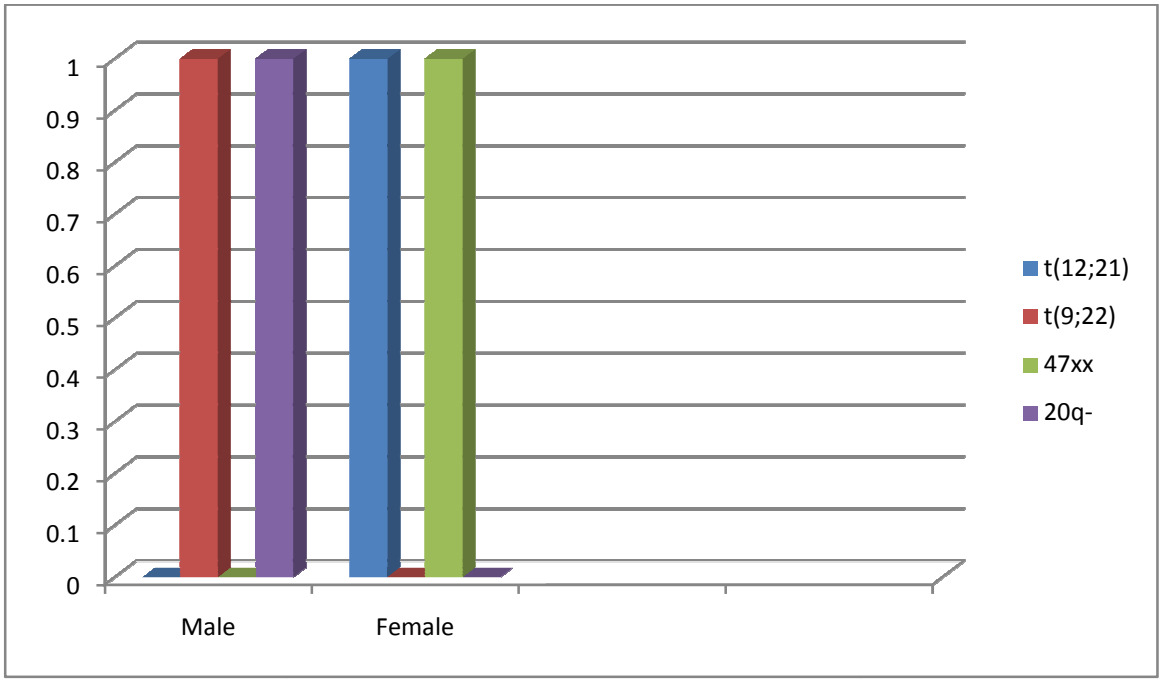


Among the 4 cases of ALL, there was an equal frequency of distribution among males and females. The following table illustrates the karyotype with the sex distribution.

Table 8: Sex distribution of karyotype in ALL

Sex	47xx	t(9;22)	t(12;21)	20q-	%
Male	0	1(25%)	0	1(25%)	50%
Female	1(25%)	0	1(25%)	0	50%

Chart 12: Sex distribution of karyotype in ALL

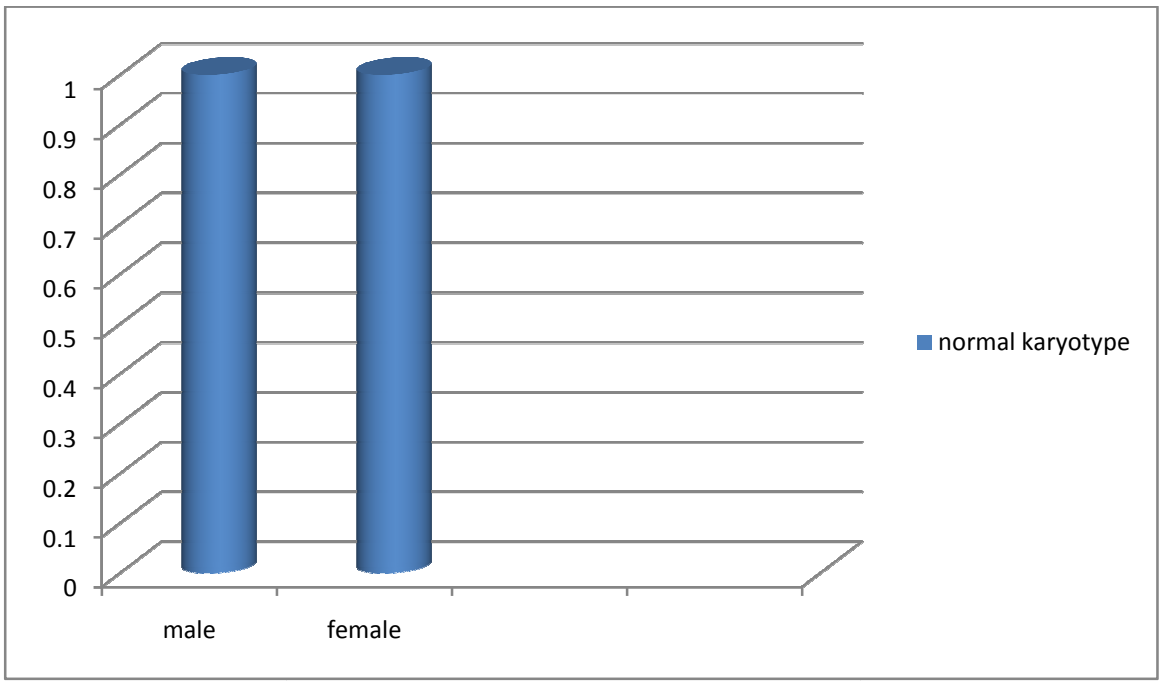


Out of the 2 cases of CLL observed, each one occurred in a male and a female patient. The following table shows the frequency distribution of karyotype observed in CLL patients among males and females.

Table 9: Sex distribution of karyotype in CLL

Sex	**normal**	**%**
Male	1(50%)	50%
Female	1(50%)	50%

Chart 13: Sex distribution of karyotype in CLL



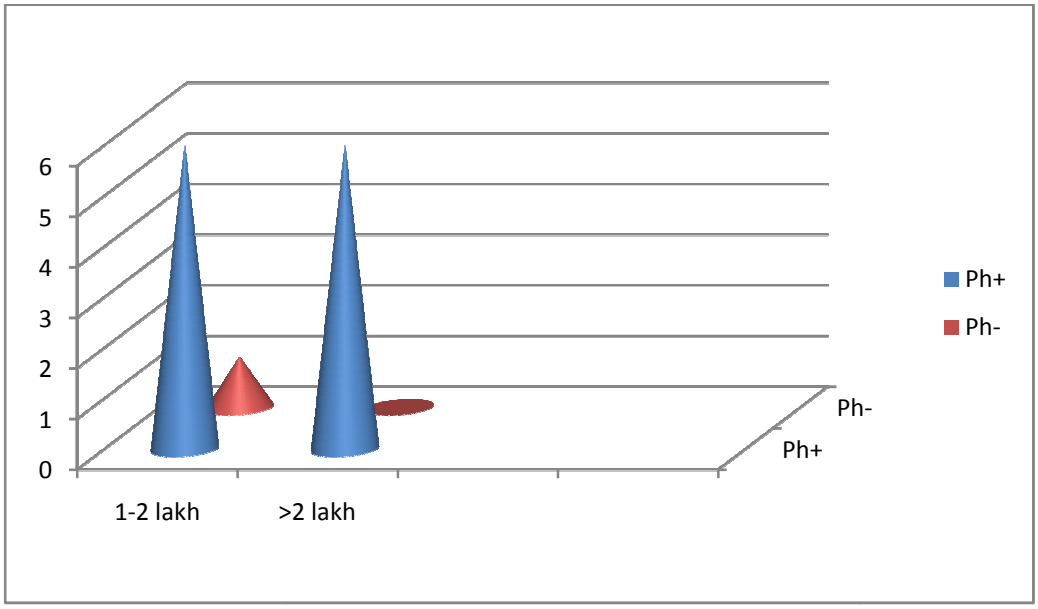
Total WBC count with karyotype in leukemia:

All the 13 cases of CML had WBC count more than 1lakh cells/mm³. Of the 13 cases, 53.84% (7 cases) had total count between 1-2 lakh/mm³, whereas 46.15% (6 cases) had total leucocyte count more than 2 lakh/mm³. The following table compares the total WBC counts with the karyotype in CML.

Table 10: Total WBC count with karyotype in CML

WBC count/mm³	Ph+	Ph-	%
1-2 lakh	6(46.15%)	1(7.69%)	53.84%
>2 lakh	6(46.15%)	0	46.15%

Chart 14: Total WBC count with karyotype in CML

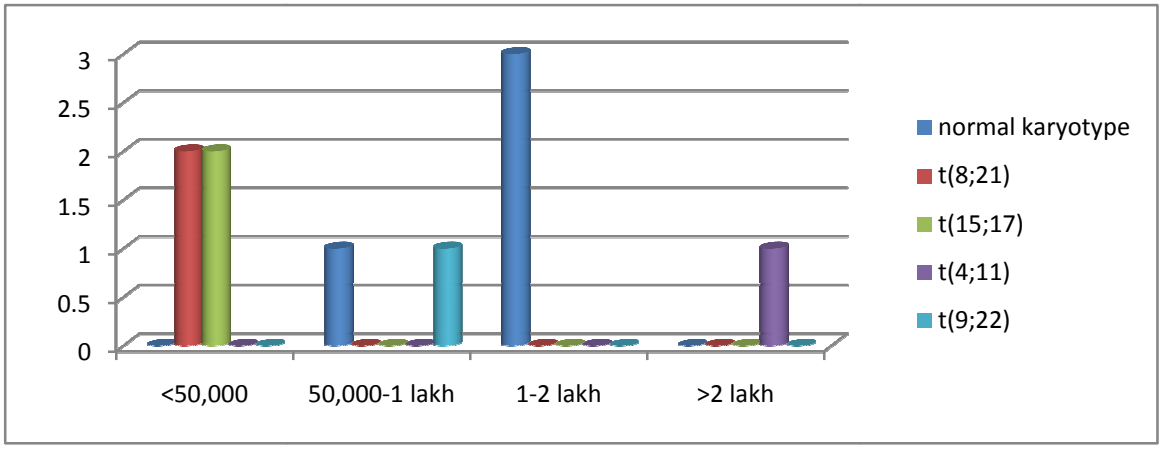


Among the 10 cases of AML, 40%(4 cases) had total count less than 50,000 cells/mm³, 30%(3 cases) had total count between 1-2 lakh cells/mm³, 20%(2 cases) had counts between 50,000 to 1 lakh/mm³. 10% of the cases had count more than 2 lakh cells/mm³. The following table illustrates the comparison of total count with various karyotypes.

Table 11: Total WBC count with karyotype in AML

WBC count/mm³	Normal	t(8;21)	t(15;17)	t(4;11)	t(9;22)	%
50,000	0	2(20%)	2(20%)	0	0	40%
50,000 to 1 lakh	1(10%)	0	0	0	1(10%)	20%
1-2 lakh	3(30%)	0	0	0	0	30%
>2 lakh	0	0	0	1(10%)	0	10%

Chart 15: Total WBC count with karyotype in AML



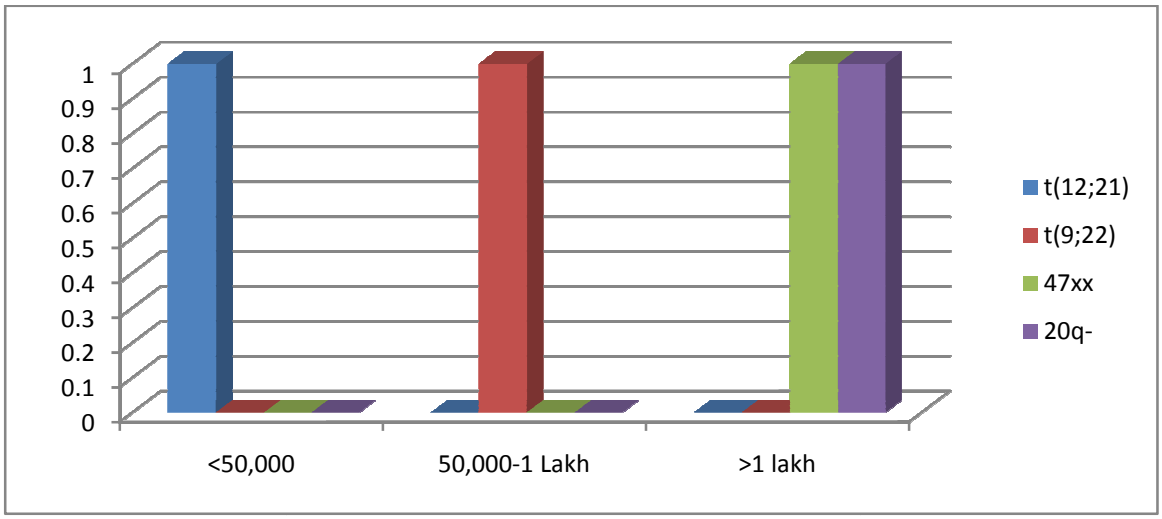
In count of <50,000 cells/mm³, there was an equal distribution of 20%(2 cases) each of karyotypes t(8;21) and t(15;17). 3 cases out of the 4 cases of normal karyotype had total counts between 1-2 lakh cells/mm³.

Among the 4 cases of ALL, 50%(2 cases) had leucocyte counts between 1-2 lakh/mm³,whereas 25% (1 case) had t(12;21) with WBC count <50,000 cells/mm³ and 25%(1 case) had t(9;22) with leucocyte count between 50,000- 1 lakh cells/mm³.

Table 12: Total WBC count with karyotype in ALL

WBC count/mm³	47xx	t(9;22)	t(12;21)	20q-	%
50,000	1(25%)	0	0	0	25%
50,000 to 1 lakh	0	1(25%)	0	0	25%
1-2 lakh	0	0	1(25%)	1(25%)	50%

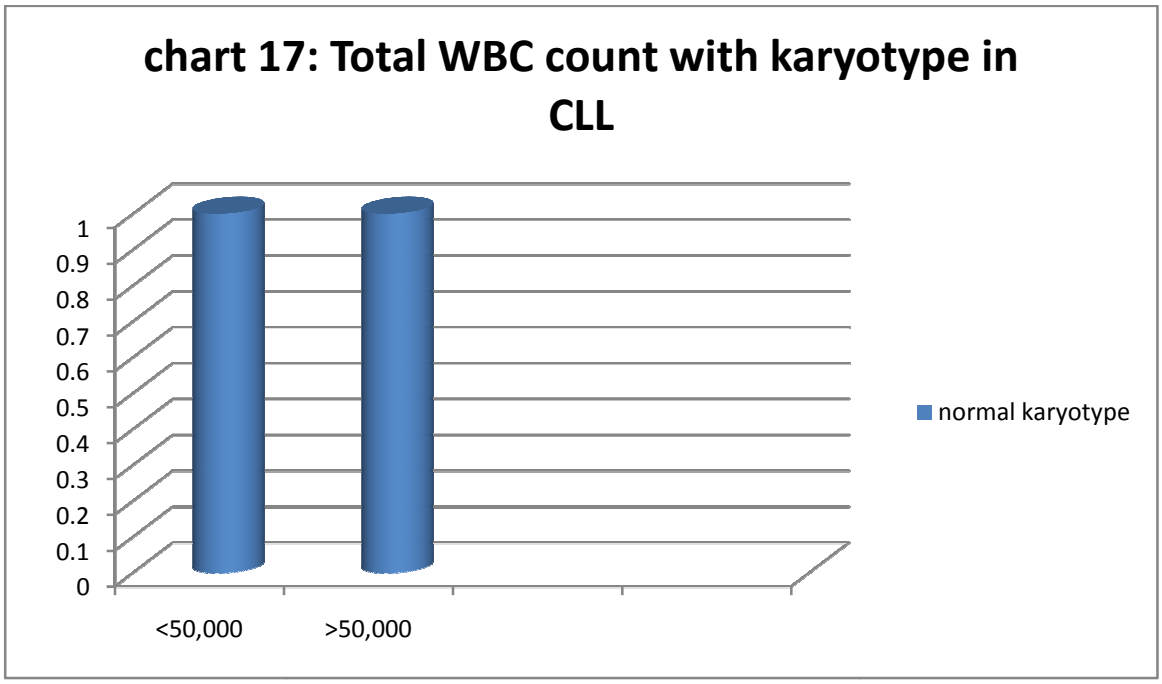
Chart 16: Total WBC count with karyotype in ALL



Among the 2 cases of CLL, 50% had leucocyte count <50,000cells/mm³ and other 50% had count >50,000 cells/mm³.

Table 13: Total WBC count with karyotype in CLL

WBC count/mm³	Normal karyotype	%
<50,000	1(50%)	50%
>50,000	1(50%)	50%



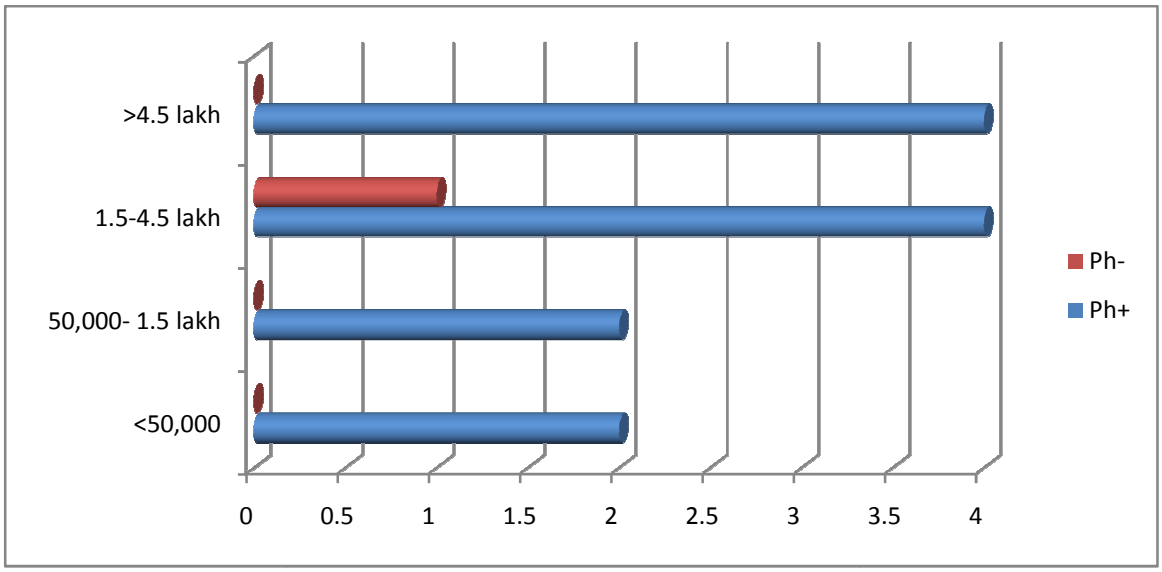
Platelet count with karyotype in leukemia:

In CML, 38.46%(5 cases) had normal platelet counts, 30.76%(4 cases) had increased platelet counts, 30.76%(4 cases) had reduced platelet count. The following table illustrates the distribution of karyotypes with platelet counts.

Table 14: Platelet count with karyotype in CML

Platelet count cells/mm³	Ph+	Ph-	%
<50,000	2(15.38%)	0	15.38%
50,000- 1.5 lakh	2(15.38%)	0	15.38%
1.5-4.5 lakh	4(30.76%)	1(7.69%)	38.46%
>4.5 lakh	4(30.76%)	0	30.76%

Chart 18: Platelet count with karyotype in CML

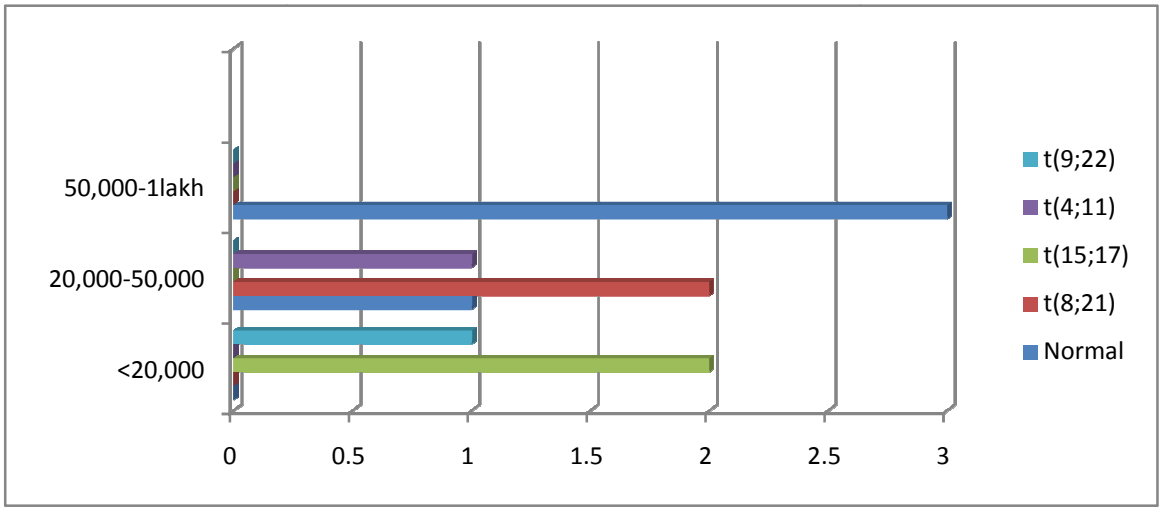


In the 10 cases of AML, 40% (4 cases) had platelet counts between 20,000 and 50,000 cells/mm³, 30% (3 cases) had platelet count <20,000 cells/mm³, of which 2 cases had t(15;17) and 30% (3 cases) had counts between 50,000 and 1 lakh cells/mm³, of which all of them had normal karyotypes.

Table 15: Platelet count with karyotype in AML

Platelet count/mm³	Normal	t(8;21)	t(15;17)	t(4;11)	t(9;22)	%
<20,000	0	0	2(20%)	0	1(10%)	30%
20,000-50,000	1(10%)	2(20%)	0	1(10%)	0	40%
50,000-1lakh	3(30%)	0	0	0	0	30%

Chart 19:Platelet count with karyotype in AML

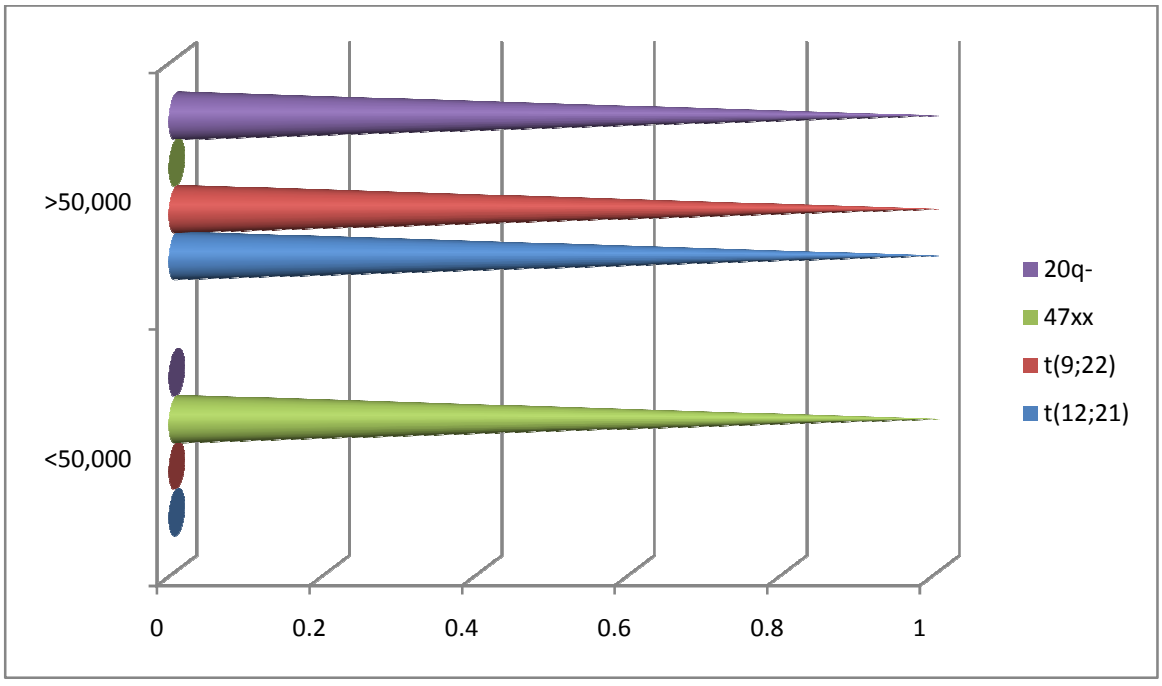


3 cases of ALL had platelet count of more than 50,000 cells/mm³ contributing to 75%, whereas 1 case had count of less than 50,000 cells/mm³. The following table denotes the distribution of platelet counts observed among ALL patients with various karyotypes.

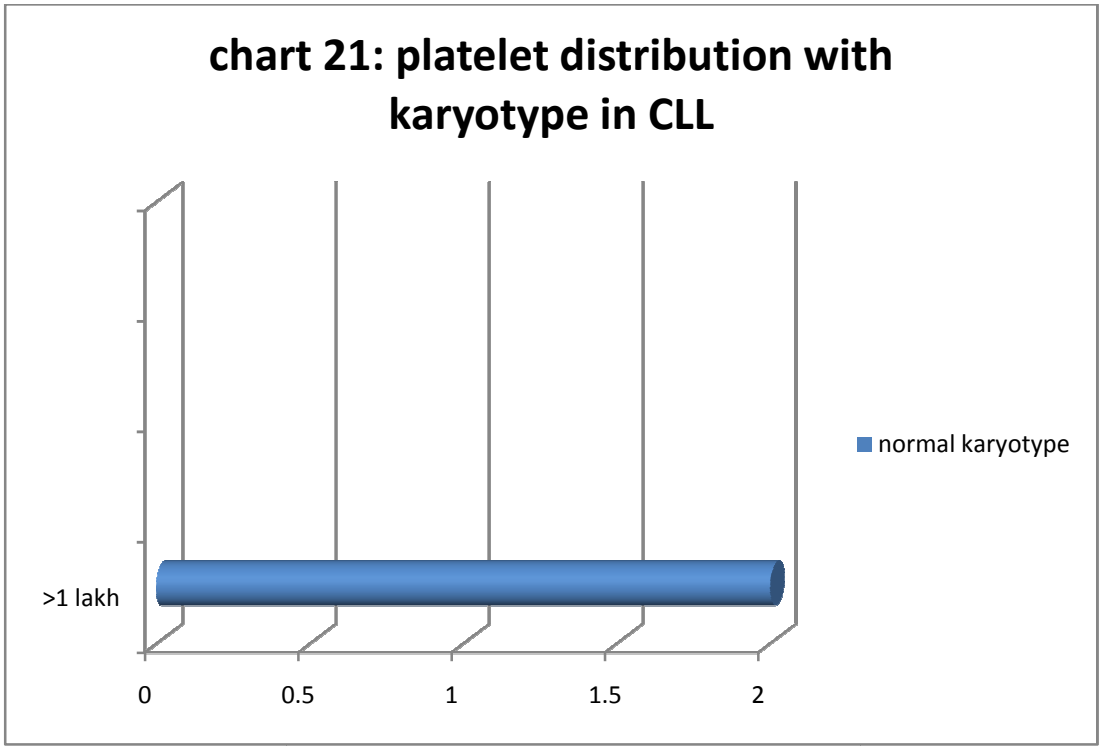
Table 16: Platelet count with karyotype in ALL

Platelet count/mm³	t(12;21)	t(9;22)	47xx	20q-	%
<50,000	0	0	1(25%)	0	25%
>50,000	1(25%)	1(25%)	0	1(25%)	75%

Chart 20 : Platelet count with karyotype in ALL



Both cases of CLL in the study had >1 lakh cells/mm³. The chart denotes the distribution of platelet count with the karyotype.



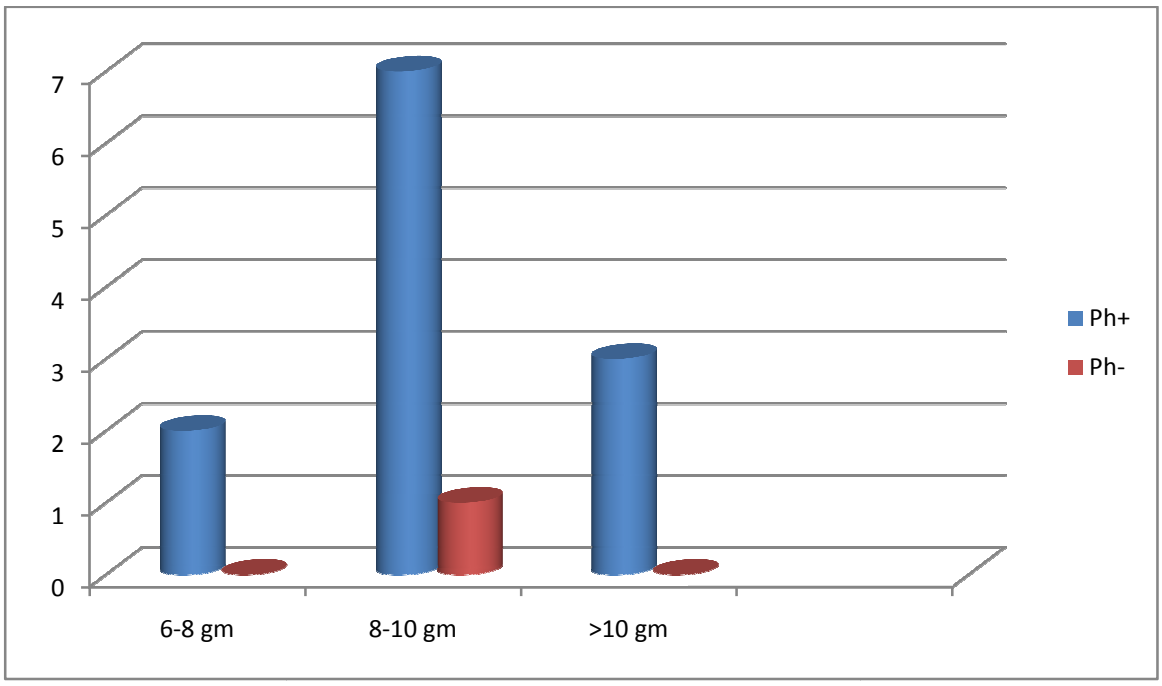
Hemoglobin with Karyotypes in leukemia:

The majority of 61.53% (8 cases) had hemoglobin level between 8 and 10, of which 7.69% (1 case) was Ph- and the remaining 53.84% (7 cases) were Ph+. The following table denotes the distribution of hemoglobin levels with the karyotypes in CML.

Table 17: Hemoglobin level with karyotype in CML

Hemoglobin gm/dl	Ph+	Ph-	%
6-8	2(15.38%)	0	15.38%
8-10	7(53.84%)	1(7.69%)	61.53%
>10	3(23.07%)	0	23.07%

Chart 22: Hemoglobin level with karyotype in CML

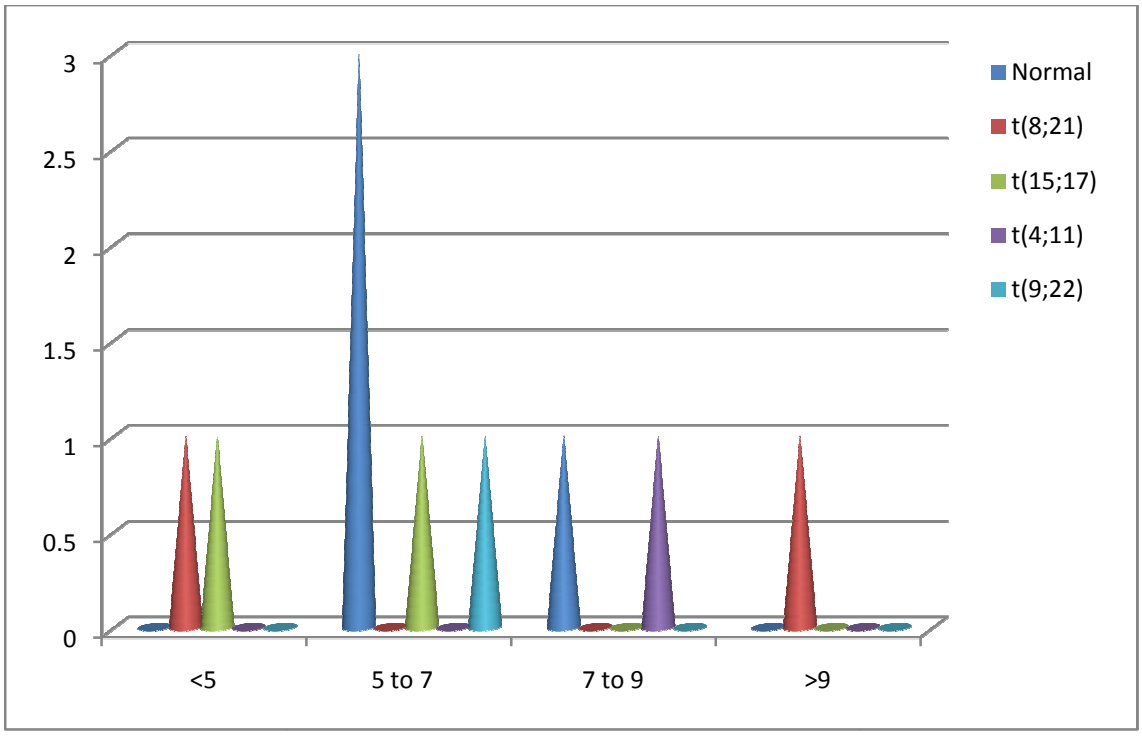


Majority of the AML patients had hemoglobin levels between 5and 7, of which 30% (3 cases) had normal karyotype, 10% had t(15;17) and 10% had t(9;22). The following table denotes the hemoglobin levels with karyotypes observed in AML patients.

Table 18: Hemoglobin level with karyotype in AML

Hemoglobin gm/dl	Normal	t(8;21)	t(15;17)	t(4;11)	t(9;22)	%
<5	0	1(10%)	1(10%)	0	0	20%
5-7	3(30%)	0	1(10%)	0	1(10%)	50%
7-9	1(10%)	0	0	1(10%)	0	20%
>9	0	1(10%)	0	0	0	10%

Chart 23: Hemoglobin level with karyotype in AML

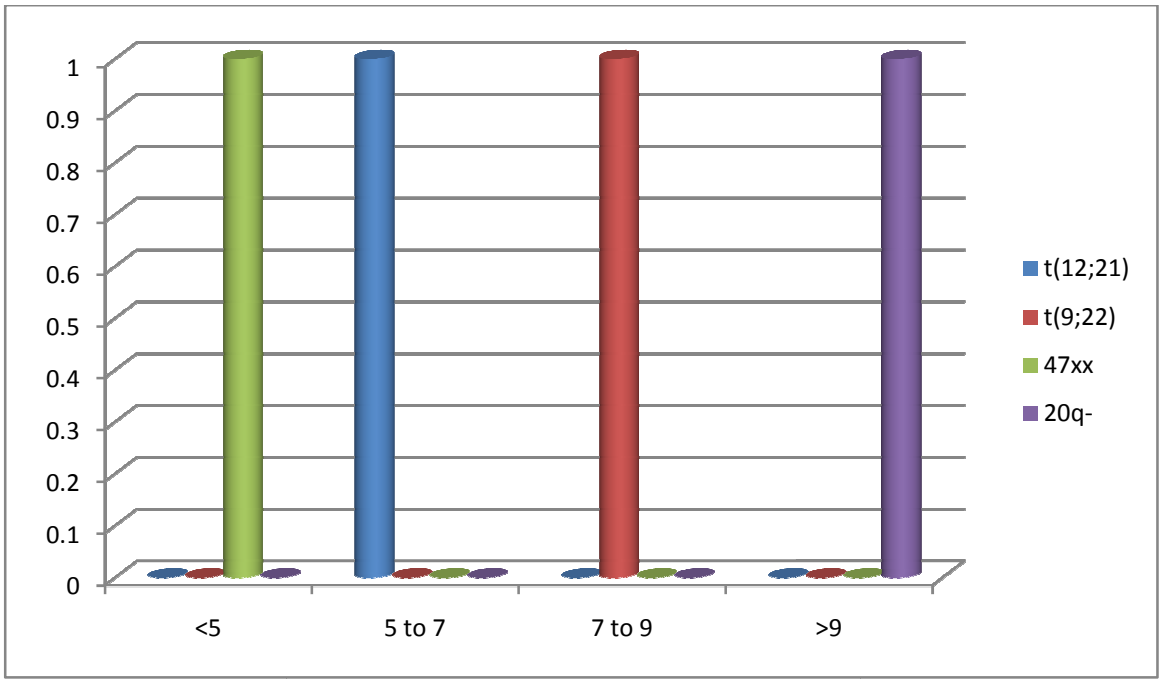


Among the 4 cases of ALL, all the 4 cases had different hemoglobin levels with different karyotypes in each. The following table denotes the hemoglobin levels with karyotypes observed in ALL patients.

Table 19: Hemoglobin level with karyotype in ALL

Hemoglobin gm/dl	t(12;21)	t(9;22)	47xx	20q-	%
<5	0	0	1(25%)	0	25%
5-7	1(25%)	0	0	0	25%
7-9	0	1(25%)	0	0	25%
>9	0	0	0	1(25%)	25%

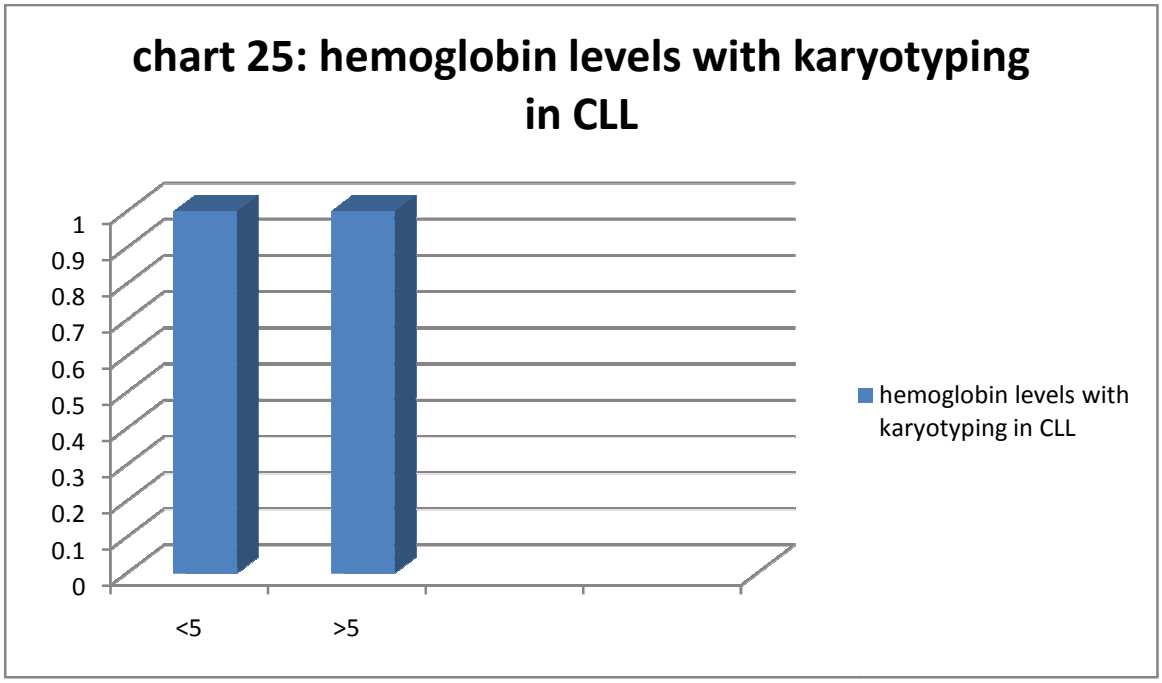
Chart 24: Hemoglobin level with karyotype in ALL



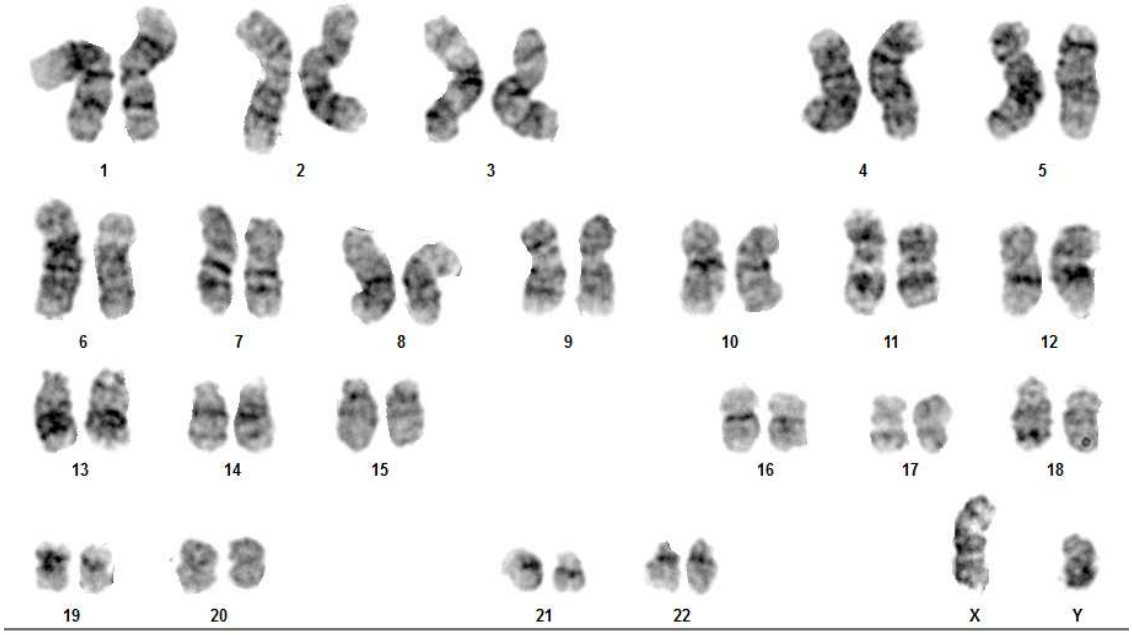
Of the 2 cases of CLL, both had normal karyotype with 50% (1case) had hemoglobin level less than 5gm/dl, whereas 50% (1 case) had hemoglobin level more than 5gm/dl. The following table denotes the hemoglobin levels with karyotypes observed in CLL patients.

Table 20: Hemoglobin level with karyotype in CLL

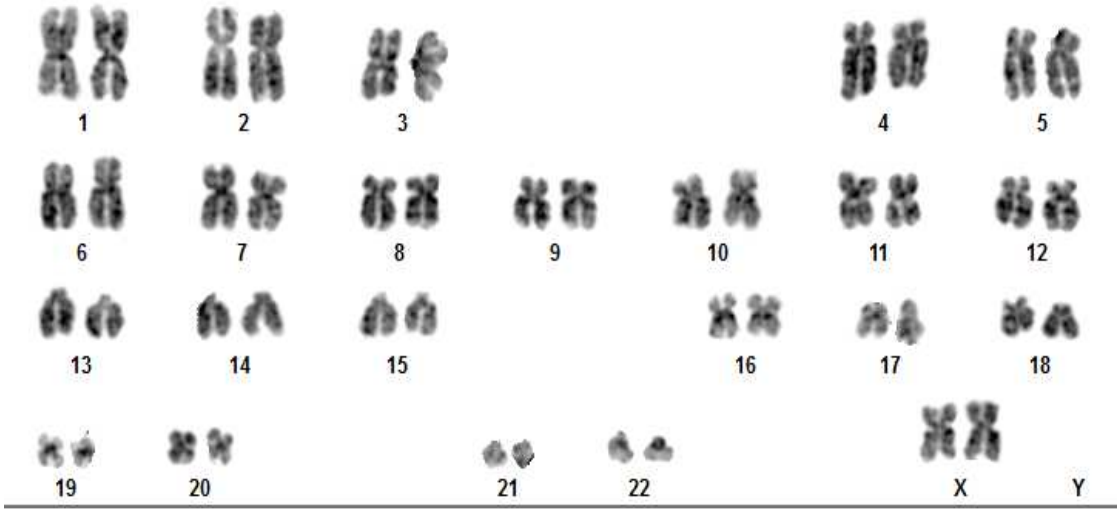
Hemoglobin gm/dl	Normal karyotype	%
<5	1(50%)	50%
>5	1(50%)	50%



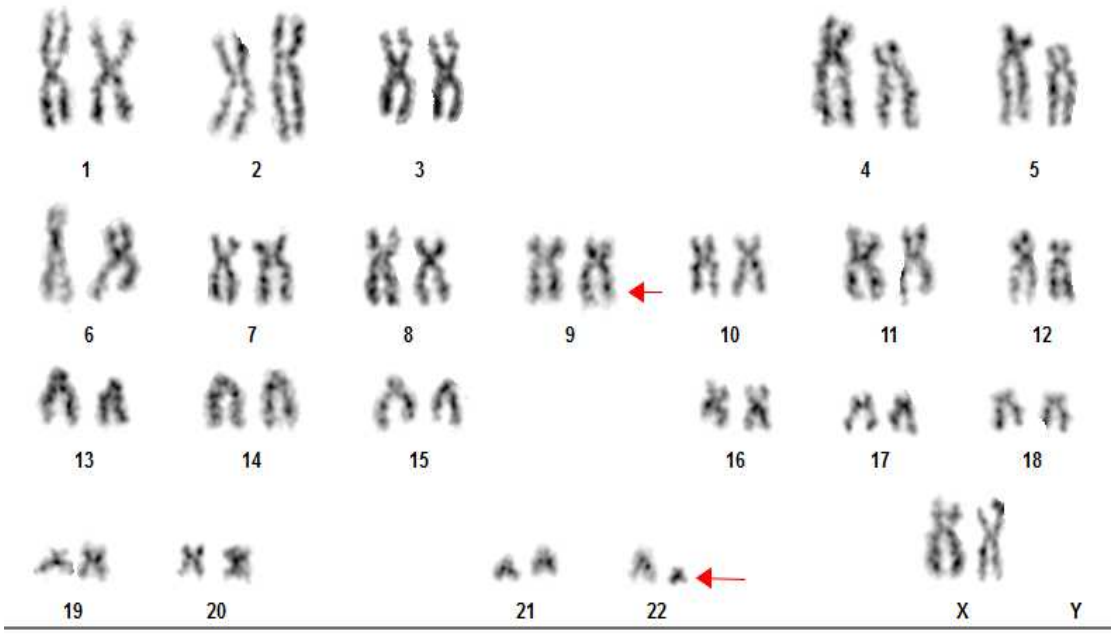
COLOUR PLATES



Normal male karyotype



Normal female karyotype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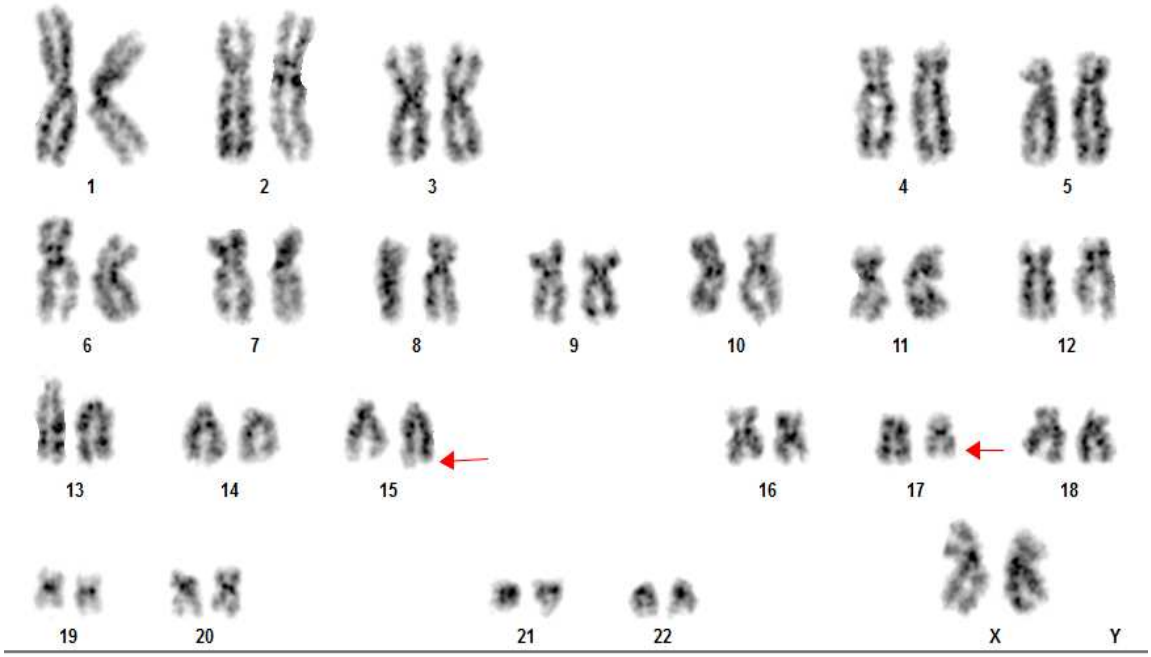
t(9;22)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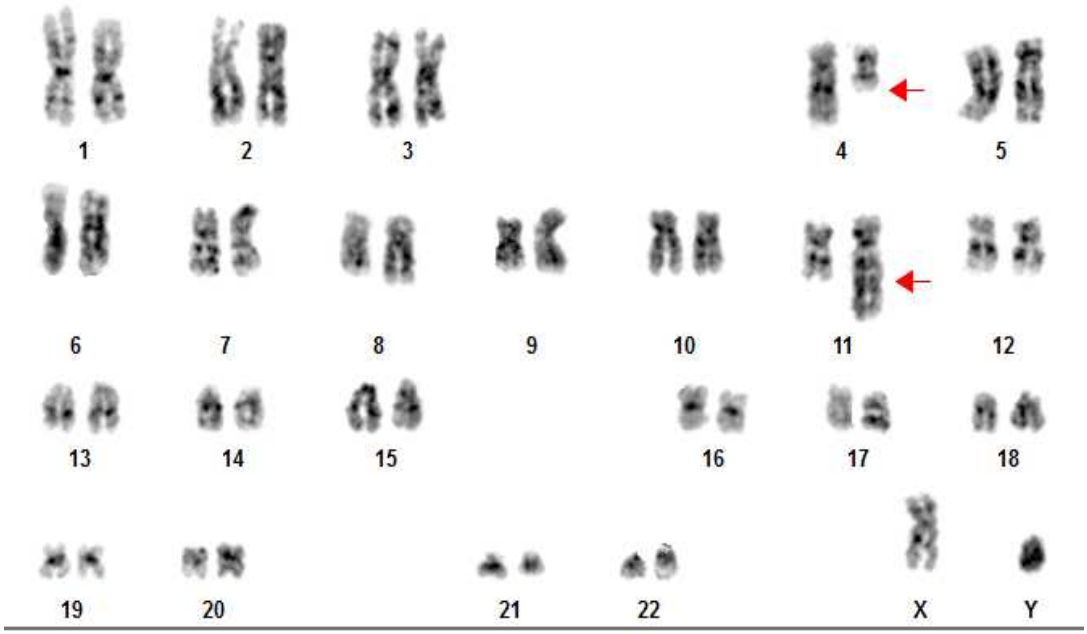
t (15;17)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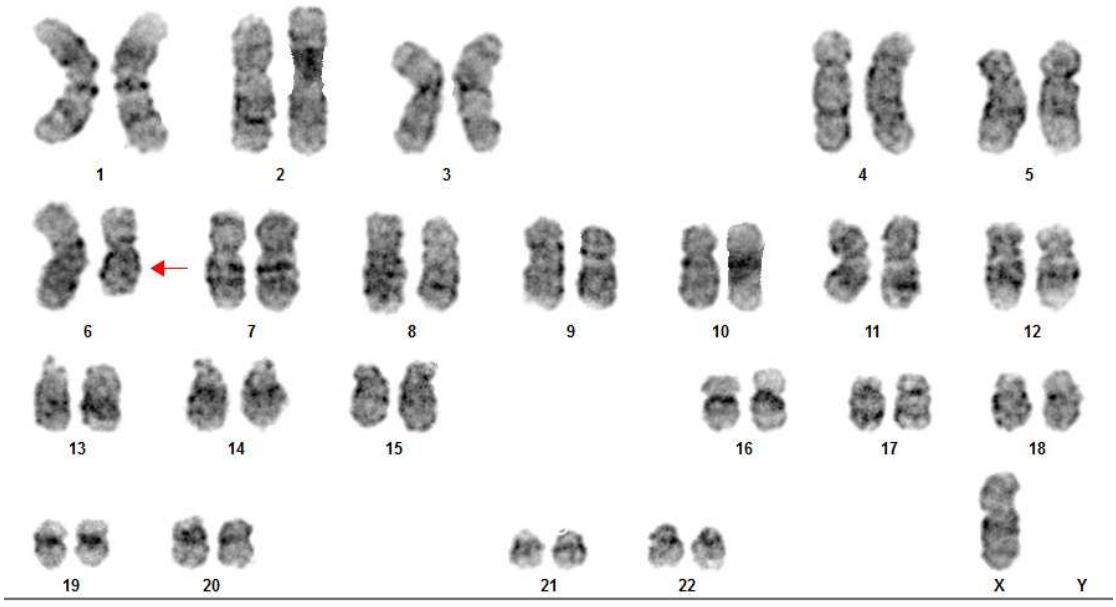
t (4;11)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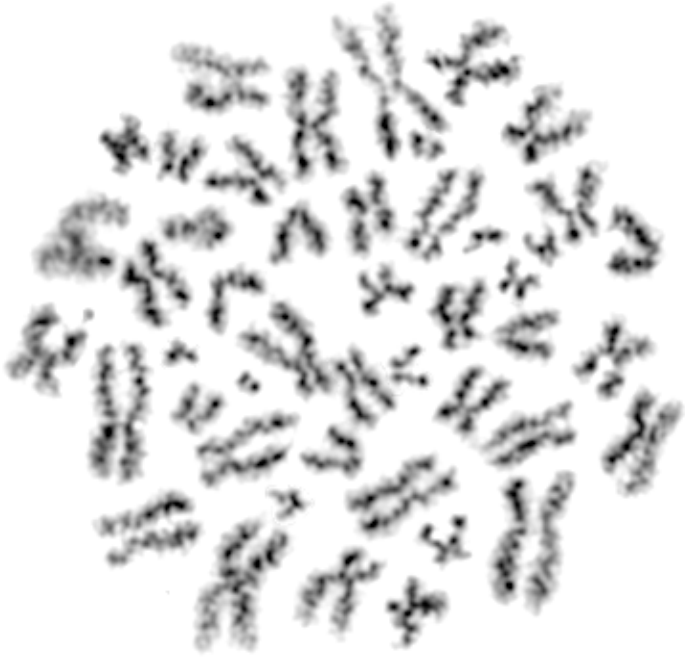
Del (6q)

GOOD METAPHASE SPREAD



POOR METAPHASE SPREAD

DISCUSSION

30 cases of leukemic patients were included in the study, with the WBC count and morphology studied by the hematology analyzer and peripheral smear stained by leishman stain. Karyotyping was done for all the 30 cases with peripheral venous blood sample.

In our study, 92.30% CML patients had Ph+ , with 7.69% had Ph-. Kaushal, S and Sidhu S.S et al. (2001) had studied 14 CML cases, in which 13(92%) patients had abnormal karyotype i.e. Ph+ chromosome. Jorge E Cortis et al. (1995) had studied 560 patients with morphologic diagnosis of CML. Cytogenetic analysis suggested that 508 (91%) cases were Ph positive. Thus, similar karyotype abnormality is observed in the present study.

The male: female ratio in CML is 1.6:1 in the present study. According to SEER program 1978-1986 for chronic myeloid leukemia, the male-to-female ratio was approximately 1.7:1. In the observations of Jose. A. Hernandez et al. (1995) the male-to-female ratio for all leukemia combined was approximately 1.7:1 for CML. In the study carried out by S.I. Sonata et al. (1978) there were 57 cases of chronic myelocytic leukemia, male to female ratio being 1.5:1. As per Jorge E Cortis et al (1995) had studied 560 patients with morphologic diagnosis of CML,

Male to female ratio was 1.3:1. C.B JHA et al. (2005) had analyzed the bone marrow samples of 19 CML cases. There were 14 male and 5 female cases and the male to female ratio was 2.8:1. This implies that the sex ratio in the present study is concordant with other studies.

The median age group in the present study is 46.5 years. According to SEER program 1978-1986 for chronic myeloid leukemia, the median age at diagnosis was 65 years. In the study carried out by S.I. Sonata et al. (1978), of the 57 cases of chronic myelocytic leukemia, the patients were in the age group of 20-68 years. The median age at diagnosis was 41 years. As per Jorge E Cortis et al (1995) who had studied 560 patients with morphologic diagnosis of CML. They were in the age group of 14-71 Yrs. (median age 44 Yrs.). C.B JHA et al. (2005) had analyzed 19 CML cases. They were in the age group of 15-82 years. Majority of the patients (68%) were below the age of 40 years. Our study shows corroboration with other studies.

In the present study, only 2 cases of CLL are studied and found to be cytogenetically normal. There were no structural or numerical chromosomal aberrations documented in our study among CLL patients. In the study conducted by Mishra et al 2016, there were 6 cases of CLL, of which there were 2 numerical (Trisomy 12), 1 structural t(11:14) and 1 compound 47XX t(11:14), +12 aberrations. The most frequent

chromosomal aberrations were trisomy (12) and translocation t(11:14), which consisted 75% of total chromosomal aberrations in CLL. Antonio Cuneo & Massimo Balboni (1995) studied 42 CLL cases diagnosed by FAB classification. There were 7 cases of translocations t(11:14) six cases of trisomy (12) and 11 cases had other recurring clonal abnormalities. Cytogenetic diagnosis reveals t(11:14) which was typical in CLL. Alain-Delmeret al. (1995) analysed 32 patients in which 28 patients showed abnormal karyotypes. Translocation t(11:14) (q13 q32) and del (11q13) was found in 18(56%) patients. The other aberrations were chromosome 11q13 either a t(11:14) or a del (11) (q13) without evidence of chromosome 14 involvement and concluded that t(11:14) was the most common translocation in CLL. As per the observation of Martin Schorder et al. (1995) seventy patients of CLL were analysed cytogenetically. The chromosomal aberrations were trisomy 12 (50%), t(11:14) 30% and others were compound translocations. Only one case had shown unbalanced 9p aberration. This shows discordance with other studies. This may be explained by the low number of CLL cases included in this study or may be due to few minor technical problems arising in karyotyping of the peripheral blood. Bernard et al (1964), Winter et al (1964) and Goh *(1967)* suggested that the apparent rarity of chromosomal abnormalities in CLL might be resulting from technical problems related to the use of peripheral blood cultures. Goh *(1967)* suggested that the low

frequency of abnormalities in CLL patients in their blood cultures might be resulting from lack of response by the leukemic lymphocytes to PHA at that time and dilution of leukemic lymphocytes by the normal lymphocytes which had responded. The normal karyotypes obtained in our study might be derived from normal lymphocyte population rather than from the leukemic cells and for that, one of the important things is the requirement of a technique that separates leukemic lymphocytes from the normal ones.

The median age group for CLL is 55.5 years in our study. In Mishra et al 2016, the mean and median age was 52.5 and 56 years respectively. Maximum numbers of CLL (66%) cases were seen in the age group of 55 to 60 years. Antonio Cuneo & Massimo Balboni (1995) studied 42 cases diagnosed by FAB classification. The median age was 60 years. (range 51-78 years). Alain-Delmer et al. (1995) analysed 32 patients with various B cell chronic lymphoproliferative disorders diagnosed pathologically. They were in the age group of 40-78 Yrs. The median age at diagnosis was 62 Yrs. there were 19 male and 13 female patients. The male to female ratio was 1.5:1. As per the observation of Martin Schorder et al. (1995), the patients in CLL were in the age of 39-81 Yrs. (median 60 Yrs). In our study, the median age is 56 years, which is 4-6 years less than the observation of the above authors. These

variations may be due to a small group subjected for analysis or racial and geographical variation.

In this study, the male to female ratio is estimated to be 1:1. Mishra et al 2016 study revealed the male to female ratio as 2:1. In Antonio Cuneo & Massimo Balboni (1995) study, male to female ratio was 2:1. Alain-Delmer et al. (1995) analysed 32 patients with various B cell chronic lymphoproliferative disorders diagnosed pathologically. There were 19 male and 13 female patients. The male to female ratio was 1.5:1. The difference in the sex ratio is attributed to the less number of cases included in our study.

In our study of 10 AML patients in our study, 40% had normal karyotype and abnormal karyotype is present in 60%, which includes 20% of t(8;21), 20% of t(15;17), 10% each of t(9;22) and t(4;11). Zheng et al in their study of 180 AML patients had Karyotyping results of 60 AML-M2 cases, of which 22 (36.6%) had a normal karyotype, 16 patients (26.6%) with a t(8;21) translocation and 22 with other karyotypes. 3 cases showed complex karyotypes and there was a 3 patients showing a 11q23,Trisomy 8 and a chromosome 5/7 abnormality respectively. In Grimwade et al 2010, a total of 5876 AML karyotypes were analyzed and abnormalities were identified in 59% of patients. Normal karyotype was

present in 41% of patients. t(8;22) contributed to 7% and t(15;17) contributed to 14%, other numerical and structural aberrations contributing for the remaining. Tong et al, in their study of 192 Chinese patients with AML, 9 cases of AML-M1 of which 7 cases (77.7%) showed a normal karyotype and 2 with other karyotypes.

The incidence of normal karyotype obtained in AML patients in our study is in concordance with other studies, as listed above. The difference in the incidence of other karyotypes in our study with other studies may be explained on the basis of ethnic, genetic and other environmental risk factors.

The median age of AML in our study is 43.5 years. In Mishra et al study of AML in 2016, the median age is 40 years (range: 1–79). This is strikingly different from that routinely reported in the literature from developed countries (Craig et al, 2012; Juliusson et al, 2012). This lower median age could be due to a combination tertiary centre referral bias and a different population pyramid structure in India and other developing economies, where the proportion of people over the age of 60 years is significantly lower. However, the possible role of additional genetic and environmental factors cannot be excluded.

In the present study, among AML, male to female ratio is 1:1. In Sunil et al study, males were most commonly affected contributing to

57% and the male to female was 1.38:1. Of the 93 cases, 54 (58%) were male and 39 (42%) were female. The equal male and female incidence may be attributed to the less number of cases included in the study.

All the 4 ALL cases included in our study had abnormal karyotypes. 25% cases had hyperdiploidy, other 75% was contributed by t(12;21), t(9;22) and 20q-. In Tadakal Mallana Goud et al, an abnormal karyotype was noted in 69 patients (74.2%) with either numerical or structural, or both abnormalities combined. Twenty five (26.9%) patients showed numerical abnormalities only, thirty six (38.7%) patients showed structural abnormalities only, eight (8.6%) patients showed both. Douet-Guilbert et al., 2003 has shown the translocation, t(12;21)(p13.3;q22), to be the most frequent, but cytogenetically largely undetected chromosomal anomaly in childhood ALL, occurring in 25-30% patients. This shows concordance with our study in which t(12;21) contributes to 25%. Moorman et al 2010 has shown the t(9;22)(q34.1;q11.2) in 30% of adults. In our present study, t(9;22) accounts for 25%.

The median age group of ALL cases in our study is 18.5 years. In Tadakal Mallana Goud et al, median age of Adult ALL patients at diagnosis was 17.5 years (range 13 to 22 years). Kaushal et al 2002, the mean age group was 19.16 years (range 3.5 – 42 years). The age distribution in our study corroborates with other studies listed here.

In the present study, male to female ratio is 1:1. In Tadakal Mallana Goud et al, The incidence was significantly higher in males (58%) than in females (42%), contributing to the male to female ratio of 1.38:1. In Kaushal et al, the male to female ratio was 9:1. This great difference in the sex ratio is attributed by the children with ALL less than 10 years included in the Kaushal et al study.

SUMMARY

Most of the CML cases in this study shows the classical Philadelphia chromosome. AML cases have shown normal karyotypes and translocations t(8;21), t(15;17) and t(9;22). ALL cases have shown the translocations t(12;21) and numerical aberrations. CLL cases have shown normal karyotype. Karyotyping in leukemia, both acute and chronic carries prognostic significance in most of the subtypes; in few cases, they confer a therapeutic significance in some types of leukemia. The culture setting with peripheral blood seems a difficult task, as most of the studies have shown lots of culture failures with peripheral blood and bone marrow aspirate culture for yielding better metaphases. Older age group generally favours CML. CLL is also common in older age group, whereas AML is common in middle age group and ALL being more common in younger age group, less than 20 years. The sex distribution is equal among both male and female in cases of AML, ALL and CLL. CML shows a male preponderance with ratio of 1.6:1.

CONCLUSION

Conventional cytogenetics is a cost-effective, simple technique that allows an overall view of the genome. This can be combined with other molecular methods like florescent in situ hybridization (FISH), which is essential to enable the detection of cryptic rearrangements and PCR, which helps in quantification of the mutated protein. Spectral karyotyping involves study of the whole genome. The recent WHO 2016 classification of hematolymphoid malignancies highlights the significance of molecular testing in leukemia. Only a few cases of leukemia are included in this study. This is due to financial constraints and this is one of the major limitation of the study. Further, peripheral smear is used for karyotyping in the present study, which again is a major limiting factor as the yield of metaphases are quite few. Thus, cytogenetic studies are warranted in larger groups of hematologic malignancies, with the bone marrow sample, so that it may contribute to diagnosis and prognosis and thus, increase the utility of cytogenetics in the development of targeted therapeutic drugs against specific mutated genes or proteins.

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ANNEXURES

INFORMATION SHEET

- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

- The purpose of this study is to plan for appropriate treatment measures in cases of specific karyotypic abnormalities easily with the help of karyotype by G-Band analysis.

- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

- We are conducting a study on role of chromosomal abnormalities in leukemic patients attending Government General Hospital, Chennai and for that your sample may be valuable to us.

- We are selecting certain samples with increased WBC count and we may be using your sample to perform tests which in any way do not affect your final report or management.

Signature of investigator Signature of participant

Date

ஆராய்ச்சிதகவல்தாள்

ஆராய்ச்சி தலைப்பு : இரத்தப் புற்று நோய்நோயாளிகளின் மரபுத்திரிகளில் ஏற்படும் பிறழ்ச்சிகள் குறித்த ஒருஆய்வு

ஆய்வாளர் : மரு.பா.ரமேஷ்,
இரண்டாம் ஆண்டு,
நோய்குறியியல்துறை,
சென்னைமருத்துவக் கல்லூரி,
சென்னை - 600 003.

தங்களதுஇரத்தம் இங்குபெற்றுக்கொள்ளப்பட்டது.

இராஜீவ் காந்தி அரசு பொது மருத்துவமனைக்கு வரும் நோயாளிகளிடம் இருக்கும் இரத்தத்தில் ஏற்படும் புற்றுநோய் பற்றி ஒரு ஆராய்ச்சி இங்கு நடைபெற்று வருகின்றது.

இரத்தத்தில் ஏற்படும் புற்றுநோய்க்கான குரோமோசோம்களில் ஏற்படும் மாற்றங்களை கண்டறிவதே இந்த ஆய்வின் நோக்கமாகும்.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இந்த ஆராய்ச்சியில் உங்களுடைய இரத்தத்தில் சில சிறப்புப் பரிசோதனைக்கு உட்படுத்தி அதன் தகவல்களை ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்குள்ளாகாது என்பதையும் தெரிவித்துக் கொள்கிறோம்.

முடிவுகளைஅல்லது கருத்துகளை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில்தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்தஆ ராய்ச்சியில் இருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்தசிறப்புப் பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்தஆய்வை பற்றிய சங்தேகங்களுக்கு தொடர்பு கொள்ள வேண்டியவர் மரு.பா.ரமேஷ், செல்: 9943495908

பங்கேற்பாளர் கையொப்பம்.....இடம்:.....தேதி:.....

பங்கேற்பாளர் பெயர் மற்றும் விலாசம்.....

ஆராய்ச்சியாளர் கையொப்பம்.....இடம்:.....தேதி:.....

INFORMED CONSENT FORM

Title of the study: " **A STUDY OF CHROMOSOMAL ABNORMALITIES IN LEUKEMIC PATIENTS IN A TERTIARY CARE HOSPITAL**"

Name of the Participant :

Name of the Principal (Co-Investigator) :

Name of the Institution : MadrasMedicalCollege

Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in "**A STUDY OF CHROMOSOMAL ABNORMALITIES IN LEUKEMIC PATIENTS IN A TERTIARY CARE HOSPITAL**"

1. I have read and understood this consent form and the information provided to me.

2. I have had the consent document explained to me.

3. I have been explained about the nature of the study in which the blood sample will be tested for chromosomal analysis by karyotyping.

4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.

5. I have informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.

6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

7. I have understand that my identity will be kept confidential if my data are publicly presented

8. I have had my questions answered to my satisfaction.

9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name_____ Signature_____

Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name_____ Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name_____ Signature_____

Date_____

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு : இரத்தப் புற்றுநோய் நோயாளிகளின் மரபுத்திரிகளில் ஏற்படும் பிறழ்ச்சிகள் குறித்த ஒருஆய்வு

சென்னை மருத்துவக் கல்லூரி நோய் குறியல்துறையில் மரு.பா.ரமேஷ் அவர்கள் மேற்கொள்ளும் இந்த ஆய்வில்பங்குகொள்ள ..... ஆகிய நான் முழு மனதுடன் சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்துகொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்ப்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில்தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும் அதனால் எந்தபாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்துகொண்டேன்.

நான் இரத்தத்தில் ஏற்படும் புற்றுநோயினை குறித்த இந்த ஆராய்ச்சியின் விவரங்களைக் கொண்ட தகவல்தாளைப் பெற்றுக் கொண்டேன்.

நான் என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக் கொள்ள சம்மதிக்கிறேன்.

எனது இரத்தத்தில் குரோமோசோம் ஆராய்ச்சி மற்றும் சிறப்பு பரிசோதனை செய்து கொள்ள சம்மதம் தெரிவிக்கிறேன்.

பங்கேற்பாளர் கையொப்பம்.....இடம்:.....தேதி:.....

பங்கேற்பாளர் பெயர் மற்றும் விலாசம்.....

ஆராய்ச்சியாளர் கையொப்பம்.....இடம்:.....தேதி:.....

MASTERCHART

S no	Age	Sex	Tc	Hb	Platelet	Karyotype	Diagnosis
1	45	M	1,33,000	6.8	53,000	46xy	AML
2	45	M	2,21,400	9.5	2,82,000	46xy, t(9;22)(q34;q11.2)	CML
3	45	M	1,23,000	9.2	1,40,000	46xy, t(9;22)(q34;q11.2)	CML
4	27	M	2,78,500	7.8	29,000	46xy, t(9;22)(q34;q11.2)	AML
5	28	F	38,200	6.1	11,000	45xx,Del(6)(q21q25)	Biphenotypic leukemia
6	32	M	1,78,280	9.8	43,000	46xy, t(9;22)(q34;q11.2)	CML chronic phase
7	70	F	1,14,400	5.7	21,000	46xx	AML
8	60	M	2,00,000	10.3	1,57,000	46xy, t(9;22)(q34;q11.2)	CML
9	20	F	1,62,000	6.7	52,000	46xx	AML
10	64	M	2,76,100	9.1	51,000	46xy, t(9;22)(q34;q11.2)	CML stable phase
11	21	F	1,48,400	4.7	14,000	47xx,+4, add(12)(q24.1)-14, +15	ALL
12	55	F	43,100	4.9	1,33,000	46xx	CLL
13	65	F	2,05,400	8.6	5,39,000	46xy, t(9;22)(q34;q11.2)	CML
14	52	F	37,820	4.5	10,000	46xy,t (15;17)	AML

15	35	M	1,11,300	9.8	73,000	45xx,Del (20q)	ALL
16	54	M	1,98,300	12.2	2,50,000	46xy, t(9;22)(q34;q11.2)	CML
17	48	F	2,78,000	6.9	40,000	46xy, t(9;22)(q34;q11.2)	CML
18	34	M	1,26,200	7.8	4,63,000	46xy, t(9;22)(q34;q11.2)	CML
19	42	M	17,600	5.0	1000	46xy,t(15;17)	AML
20	58	F	2,01,400	8.4	3,18,000	46xx, t(9;22)(q34;q11.2)	CML
21	56	F	1,30,600	9.3	6,03,400	46xx, t(9;22)(q34;q11.2)	CML
22	40	F	37,980	10.0	40,000	46xx,t(8;21)	AML
23	48	M	62,000	5.8	17,000	46xy, t(9;22)(q34;q11.2)	AML
24	38	F	1,18,000	10.5	5,10,100	46xx, t(9;22)(q34;q11.2)	CML
25	32	M	1,03,000	9.6	4,50,000	46xy,t(3,6,9,22)(p13;p21;q34;q11.2)	CML
26	23	M	24,500	4.8	27,000	46xy,t(8;21)	AML
27	14	F	29,200	6.2	65,000	46xx,t(12;21)	ALL
28	16	M	99,100	7.3	81,800	46xy,t(9;22)(q34;q11.2)	ALL
29	46	F	74,300	7.9	83,000	46xx	AML
30	56	M	68,100	5.2	1,16,000	46xy	CLL